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Low brain allopregnanolone levels mediate flattened circadian activity associated with memory impairments in aged rats

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Abstract

Background—Sleep and cognitive impairments are two of the most prevalent neuropsychiatric disorders in the aged population. Age-related memory dysfunctions can result from alterations in sleep/wake circadian rhythm. However, the underlying mechanism of these alterations is unknown. Here, we demonstrate the role of alterations in brain steroid levels in age-related sleep-dependent memory impairment in rats.

Methods—Sleep/wake circadian activity and spatial memory performance were evaluated in adult, middle-aged, and aged rats, and steroid levels were measured in brain structures involved in mediating sleep-dependent memory processes using gas chromatography/mass spectrometry. The causal relationship between circadian activity and allopregnanolone levels was assessed using an inhibitor of allopregnanolone synthesis (indomethacin).

Results—Similar to observations in humans, a subpopulation of middle-aged and aged rats show flattened amplitude of circadian activity associated with impaired spatial long-term memory performance. Sleep-dependent memory dysfunction was associated with a low level of allopregnanolone in the hypothalamus, pedunculopontine nucleus, and ventral striatum. Inhibition of allopregnanolone synthesis in young rats decreased allopregnanolone in the hypothalamus and produced flattened amplitude of circadian activity similar to aged rats.

Conclusions—These findings identify brainstem and basal forebrain allopregnanolone as an essential endogenous substrate involved in mediating sleep-dependent memory function in young and aged rats. Allopregnanolone may play a critical role in preserving individuals from age-induced alterations in sleep and memory processes and may represent a novel target for attenuating age-related declines in sleep and memory.

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Competing Interest

The authors reported no biomedical financial interests or potential conflicts of interest.

Keywords

aging; working memory; sleep; spatial; anxiety; steroids

Introduction

Population aging is one of the world's most important demographic phenomena and a critical public health problem considering that individuals over 60 years of age are at high risk for neurological and psychiatric disorders. A large body of evidence suggests that sleep and memory are two related phenomena (1,2) that are impaired during aging (3) and represent the most prevalent neuropsychiatric disorders in the aged population (between 22% to 56%) (4,5). Thus, it is of great importance to tackle the neurobiological mechanisms underlying sleep and memory impairments to develop new therapeutic strategies of aging-related behavioral deficits.

It has been proposed that a large proportion of memory impairments observed during aging are secondary to sleep fragmentation (6-8). Recent data indicate that sleep can contribute to memory through a process of memory consolidation involving off-line replay and off-line processing of information acquired while awake (9). Electrophysiological, cellular, and molecular evidence indicates that sleep-dependent memory consolidation occurs in several brain structures, including the hippocampus (10,11), ventral striatum (12), pedunculopontine nucleus (13), amygdala (10), and hypothalamus (10).

A decrease in the amplitude of the sleep/wake circadian rhythm is one of the most marked changes observed during aging, and we recently demonstrated that in aged rats the recording of locomotor activity during the circadian cycle is a good index of the sleep/wake rhythm of the individual and allows the prediction of memory performance (14). A decrease in sleep/wake circadian rhythm amplitude in aged rats is associated with a fragmentation of non-rapid-eye-movement (non-REM) sleep episodes and spatial memory impairment (14). Interestingly, similar to observations in humans, only a subpopulation of aged rats exhibits a decreased amplitude of the sleep/wake circadian rhythm. Aged rats with a decreased sleep/wake circadian amplitude (Low Amplitude [LA] animals compared with aged-matched controls) exhibit robust behavioral impairments compared with young animals, such as REM sleep fragmentation and decreased spatial memory performance, while aged rats with a high amplitude of circadian activity (HA rats) have a phenotype similar to young animals with intact sleep/wake circadian rhythm and memory function (14).

Neurosteroids are part of a class of steroids that are de novo synthesized within the nervous system independently from the steroidogenesis occurring in peripheral endocrine glands and have autocrine and paracrine actions in the central nervous system (15). Numerous pharmacological studies have shown that peripheral and central administration of allopregnanolone, pregnenolone, 3 α ,5 α -tetrahydrodeoxycorticosterone (3 α ,5 α -THDOC), testosterone, and 5 α -dihydrotestosterone (5 α -DHT) alter both sleep/wake circadian rhythms and memory performance in young animals (16-22). Administration of pregnenolone sulfate and dehydroepiandrosterone sulfate in young rats improved, whereas allopregnanolone impaired, memory in various memory tasks (22-23). As reviewed in Vallée et al., 2001, both systemic and cerebral administration routes have been used for showing the memory-enhancing effects of pregnenolone sulfate and dehydroepiandrosterone sulfate in animal studies. Few studies have investigated the effect of neurosteroids on sleep states, but administration of pregnenolone sulfate and allopregnanolone leads to opposite changes in sleep architecture and cortical activity in rodents, with pregnenolone sulfate increasing and allopregnanolone decreasing the amount of REM sleep (23). Moreover, it has been shown

that plasma and brain levels of some neurosteroids are altered during aging, especially in individual humans and animals exhibiting cognitive disorders (23-27). For example, a decrease in pregnenolone sulfate and testosterone in the brain has been observed in aged rats (22). Many conflicting studies have been reported regarding allopregnanolone, with some studies showing no change, decreases, or even increases in some brain structures, such as the hypothalamus, in aged rats (see 23 for review). This variability suggests that alterations of brain allopregnanolone levels may not be a simple consequence of aging and may depend on individual variability in cognitive functions in aged rats. Moreover, the role of endogenous neurosteroids in age-related alterations of circadian activity remains unknown.

To address this issue, we compared young, old behaviorally unimpaired (HA), and old behaviorally impaired rats (LA) for brain and plasma levels of eight steroids including pregnenolone, allopregnanolone, dehydroepiandrosterone, 3 α ,5 α -THDOC, testosterone, 5 α -DHT, pregnanolone, and epiallopregnanolone. For this study, we also extended the behavioral characterization of LA and HA animals to verify the specificity of memory impairments. Finally, because marked alterations in allopregnanolone concentration were found in the brain between impaired and unimpaired aged animals, the causal relationship between allopregnanolone levels and behavioral impairment was assessed following pharmacological blockade of allopregnanolone biosynthesis by indomethacin.

Neurosteroids were measured using a specific, sensitive, and accurate steroid quantification method based on isotope dilution combined with gas chromatography/mass spectrometry (GC/MS) (28). We focused our analysis on six different cerebral structures known to be involved in sleep-dependent memory processes: pedunculopontine nucleus, hypothalamus, dorsal striatum, ventral striatum, hippocampus, and amygdala. Accurate assessment of neurosteroid levels in small brain regions is still a challenge, and few data are currently available from studies investigating alterations in neurosteroid levels in individual brain areas with highly sensitive and specific analytical methods (29-32,28).

We found that flattened circadian activity was associated with a specific decrease in spatial long-term memory ability, without modification of working memory or other behavioral dimensions such as anxiety. Flattened circadian activity was also associated with a low level of allopregnanolone in the hypothalamus, pedunculopontine nucleus, and ventral striatum, but not the hippocampus, dorsal striatum, or amygdala. Finally, selective pharmacological blockade of allopregnanolone biosynthesis in young rats decreased allopregnanolone levels specifically in the hypothalamus and induced flattened circadian activity that was similar to aged rats.

Methods and Materials

Animals

Young (3-6-month-old, $n = 16$), middle-aged (16-18-month-old, $n = 13$), and aged (22-24-month-old, $n = 16$) male Sprague-Dawley rats (Charles River, France) were used in experiment 1. Young (3-4-month-old, $n = 24$) male Sprague-Dawley rats (Charles River, France) were used in experiment 2. Animals were housed individually under a constant 12 h/12 h light-dark cycle (lights on at 8:00 a.m.), with *ad libitum* access to food and water. They were left undisturbed until behavioral testing that was preceded by a handling phase in which animals were gently manipulated twice per day for 1 week. Temperature (22°C) and humidity (60%) were kept constant. All experiments were conducted in strict compliance with the recommendations of the European Union (86/609/EEC).

Indomethacin administration in the drinking water

Indomethacin was dissolved in 2-hydroxypropyl- β -cyclodextrin solution with a 1:2 molar ratio (33,34) and diluted with water to a final concentrations of 0.033 mg/ml (2 mg/kg/day) or 0.167 mg/ml (10 mg/kg/day) and administered in drinking water. Considering that rats were drinking an average of 65 ml/kg/day of fluid, the daily intake of indomethacin was estimated to be 2.1 ± 0.1 mg/kg/day and 10.8 ± 0.6 mg/kg/day, respectively.

Behavioral testing

Spatial Memory—Rats were tested in a water maze (180 cm $\times$ 60 cm) filled with opaque water (21°C). The procedure consisted of four phases (see Supplement 1 for details).

Habituation (1 day without platform), *Place discrimination with distal cues* (8 days with hidden platform), *Place discrimination with distal and proximal cues* (1 day with visible platform) and *Working memory* (4 days with moving hidden platform). To avoid confounding effects of swim speed on memory performance, the distance (cm) to reach the platform was calculated.

Sleep/Wake Circadian Rhythm—Locomotor activity was continuously monitored for 7 days using circular-shaped cages (60 cm diameter) equipped with infrared beams (Imétronic, France). To evaluate the integrity of sleep/wake circadian rhythm, the circadian amplitude index was calculated by dividing nocturnal activity by diurnal activity (14). This index was preferred to cosinor analysis because the circadian amplitude index, contrary to cosinor analysis, does not have the drawback of underestimating non-sinusoidal contributions to circadian locomotor waveforms (14).

Anxiety-like behavior—*Open field*: Rats were placed in an open field arena (100 $\times$ 100 $\times$ 30 cm, 55 lux) for 15 min. The number of entries onto, time spent on, and distance traveled on the central (60 $\times$ 60 cm) and peripheral (width 20 cm) areas were recorded. *Elevated plus-maze*: Rats were allowed to explore the maze for 5 min, which consisted of four arms (50 $\times$ 10 $\times$ 50 cm, 27 lux). The percentage of time spent on the open arms compared with total time on both the open and closed arms were calculated. To increase the power of analysis and reduce the number of variables, data from the elevated plus maze and open field test were subjected to a principal component analysis (35). Only the principal factor (explaining 57% of the variance) was used. Loading scores of the variables on the principal factor were the following: elevated plus maze (distance explored in open arms, -0.79 ; time spent on closed arms, $+0.82$), open-field (% time spent in the central area, -0.75 ; % time spent in the corner, $+0.64$). The loading score of each subject on the principal factor was used to compute the anxiety index.

Steroid quantification

Trunk blood was collected in EDTA-coated tubes, centrifuged at $1000 \times g$ for 10 min, and stored at -20°C . Brains were harvested (<45 s) and frozen in isopentane (-38°C). Cerebral structures were punched out at -20°C with glass Pasteur pipettes on brain slices using a cryostat following the atlas of Paxinos and Watson (36). The diameter of the glass pipettes was adapted to each structure to maximize the area and specificity of sampling (Fig. 1). Pregnenolone (3 β -hydroxypregn-5-en-20-one), allopregnanolone (3 α -hydroxy-5 α -pregnan-20-one), epiallopregnanolone (3 β -hydroxy-5 α -pregnan-ol-20-one), pregnanolone (3 α -hydroxy-5 β -pregnan-ol-20-one), tetrahydrodeoxycorticosterone (3 α ,21-dihydroxy-5 α -pregnan-20-one; 3 α ,5 α -THDOC), dehydroepiandrosterone (3 β -hydroxyandrost-5-en-17-one; DHEA), testosterone (17 β -hydroxyandrost-4-en-3-one), and 5 α -dihydrotestosterone (5 α -DHT) were determined by gas chromatography/mass spectrometry (GC/MS) according to Vallée et al. with modifications (for details, see Supplement 1 and figure 2 for a

representative chromatogram of allopregnanolone) (28). DHEA and epiallopregnanolone levels were not detectable and were excluded from the analysis.

Procedures

Experiment 1: Behavioral testing was performed at 2 pm, except for locomotor activity that was monitored for 7 consecutive days. The tests were performed in the following order: elevated plus maze, water maze, locomotor activity and open-field with 1-2 weeks between each test. Rats were sacrificed by decapitation at 2pm, 4 weeks after the last behavioral test. Experiment 2: Locomotor activity was monitored for 4 consecutive days before and after administration of indomethacin (10 mg/kg/day in drinking water for 4 days). Animals were then sacrificed immediately after the end of recording for steroid assay.

Statistical analysis

Results were analyzed with Statistica software (Statistica, USA). In all cases, a normality test and an equal variance test were performed before using Student's *t*-test or analysis of variance (ANOVA). Newman-Keul's *post hoc* test and multiple regression were used when appropriate. For ANOVAs, when normality assumptions were violated, Kruskal Wallis test was used followed by Mann-Whitney U test. Data are expressed as mean $\pm$ SEM, unless otherwise specified.

Results

Aging induces a flattened sleep/wake circadian rhythm associated with spatial long-term memory impairment but not with short-term working memory or anxiety-like behavior

The present study compared the circadian amplitude index of locomotor activity of young (3-6-month-old), middle-aged (16-18-month-old), and aged (22-24-month-old) rats. Aging was associated with a flattened circadian rhythm associated with an increase in interindividual variability. The circadian amplitude index decreased during aging ($F_{2,42} = 18.6$, $p < 0.05$, Fig. 3A), but the coefficient of variation of the population (standard deviation/mean) increased from 21.4% in the youngest group to 41.4% in the oldest group. Therefore, only a subpopulation of the 16-18 and 22-24-month-old rats exhibited a decrease of the circadian amplitude index. A subgroup of the 16-18-month-old (54%) and 22-24-month-old (88%) rats fell outside the confidence interval of values exhibited by young rats (mean $\pm$ 95% confidence interval). These animals were characterized as LA rats, whereas the remaining animals were characterized as HA rats (Fig. 3B). LA rats exhibited a 48% decrease in the circadian amplitude index compared with both young and HA rats ($F_{2,42} = 35.7$, $p < 0.05$, Fig. 4C). In contrast, HA rats exhibited a circadian amplitude index identical to young rats (mean $\pm$ SEM; young: 4.3 ± 0.2 , HA: 4.3 ± 0.3).

To characterize the relationship between the decrease in the circadian amplitude index and memory deficits, we compared spatial memory performance in young, LA, and HA rats in the spatial version of the water maze task. In this task, animals are required to locate a hidden platform using visual cues available in the testing room. Compared with the young and HA groups, LA rats exhibited impaired learning of this task, reflected by a greater distance traveled to find the hidden platform during the last 2 days of testing ($F_{2,42} = 10.0$, $p < 0.05$, Fig. 4A). This difference in performance was unlikely due to visual or motor alterations because no difference was observed between HA and LA rats in the visual discrimination task ($F_{2,42} = 1.9$, not significant, Fig. 4B). Moreover, LA rats did not exhibit any alterations in working memory in the water maze. All groups exhibited similar performance (Fig. 4C), demonstrated by a similar decrease in the distance to find the hidden platform across the four successive daily trials (trial $\times$ group interaction, $F_{6,126} = 0.8$, not significant) when the working memory version of the water maze was used.

To further characterize the specificity of the relationship between the circadian amplitude index and memory performance, we evaluated anxiety-like behavior in young, LA, and HA rats. Anxiety-like behavior measured in the open field and elevated plus maze tests differed with age but were not associated with circadian rhythm impairment. The index of anxiety increased during aging ($F_{2,42} = 12.5$, $p < 0.05$, Fig. 4D) similarly in HA and LA rats. Moreover, a multiple regression analysis using spatial memory as the dependent variable and age, circadian amplitude index, visual discrimination, working memory, and anxiety-like behavior as predictors demonstrated that only the circadian amplitude index was significantly correlated with spatial memory ($F_{1,38} = 12.97$, $\beta = -0.51$, $B = -1.7$, $p < 0.001$).

LA animals exhibit decreased brain levels of allopregnanolone

To attempt to correlate modifications of brain neurosteroid content with variations in circadian amplitude during aging, we assessed steroid levels in six brain structures known to be involved in sleep-dependent memory processes in young, LA, and HA rats.

Allopregnanolone levels differed between young, HA, and LA rats in the pedunculopontine nucleus, hypothalamus, and ventral striatum ($H_{2,42} > 6.1$, all $p < 0.05$, Fig. 5A-C). In these structures, LA rats exhibited lower concentrations compared with HA rats (Mann-Whitney U test: $Z > 2.1$, $p < 0.05$), whereas HA rats exhibited similar concentrations (hypothalamus: Mann-Whitney U test: $Z = 1.3$, not significant; ventral striatum: Mann-Whitney U test: $Z = 1.4$, not significant), or higher concentrations (pedunculopontine nucleus: Mann-Whitney U test: $Z > 2.1$, $p < 0.05$) than young rats. No statistically significant differences in allopregnanolone levels were found in the hippocampus, dorsal striatum, and amygdala between HA and LA rats ($H_{2,42} < 2.3$, not significant; Mann-Whitney U test: all $Z < 1.3$, not significant, Fig. 5D-F). An increase in allopregnanolone was found in plasma in both HA and LA rats compared with young rats ($H_{2,29} > 7.1$, $p < 0.05$, Fig. 5M). In contrast, no difference between the two groups was found in the concentration of the steroid precursor pregnenolone for any structures analyzed ($H_{2,42} < 4.3$, not significant, Fig. 5G-L). Despite very low levels of pregnenolone in plasma, we found that LA rats exhibited an increase of pregnenolone concentration compared with HA and young rats ($H_{2,26} = 12.4$, $p < 0.05$, Mann-Whitney U test: $Z = 2.7$, $p < 0.05$, Fig. 5N). Additionally, concentrations of testosterone, 5 α -DHT, 3 α ,5 α -THDOC, epiallopregnanolone, DHEA, and pregnanolone in the different structures and plasma did not differ between HA and LA rats (see Supplement 1, with the exception of DHEA because DHEA levels were undetectable in all conditions).

Altogether, these data show that during aging the levels of allopregnanolone tend to increase in most brain structures studied. However, in LA rats, either this increase is flattened or, as observed in the hypothalamus, allopregnanolone levels actually decrease even when compared with young animals.

Blockade of allopregnanolone synthesis in young rats decreases allopregnanolone level in the hypothalamus and induces flattened amplitude in circadian activity

To evaluate the possible causal relationships between allopregnanolone levels and the flattened circadian activity observed in LA animals, we tested in young rats the effect of chronic oral administration of indomethacin, a potent inhibitor of 3 α -hydroxysteroid oxidoreductase (3 α -HSOR cytosolic form) (37), which catalyzes the biosynthesis of allopregnanolone from 5 α -dihydroprogesterone. Indomethacin was administered in drinking water for 4 days at 10 mg/kg/day, a dose known to inhibit the synthesis of allopregnanolone without affecting general health in rats (38). Oral administration of indomethacin dose-dependently decreased the circadian amplitude index by 33% compared with the vehicle-treated group ($F_{2,14} = 3.9$, $p < 0.05$, Fig. 6) and decreased allopregnanolone levels by 57% specifically in the hypothalamus (control 10 ± 1.7 fmol/mg protein vs. indomethacin $4.3 \pm$

1.5 fmol/mg protein, $t_{1,14} = 1.35$, $p < 0.05$) without affecting allopregnanolone levels in the pedunculo pontine nucleus, dorsal striatum, ventral striatum, amygdala, and hippocampus (all $p > 0.05$). The indomethacin-induced decreases in circadian amplitude index (-33% vs. control) and allopregnanolone level in the hypothalamus (-57% vs. control) observed here in young rats were similar to the decreases observed in old impaired LA rats (circadian amplitude index: -47% vs. HA rats; hypothalamus allopregnanolone level: -49% vs. HA rats), suggesting that pharmacological inhibition of allopregnanolone biosynthesis in young rats mimicked part of the deficits observed in LA rats during aging.

Discussion

This study extends our understanding of age-related behavioral impairments by showing that changes in neurosteroid levels in specific brain regions are associated with these dysfunctions. Rats with a flattened circadian amplitude index and spatial long-term memory impairment (LA animals) exhibited decreased levels of allopregnanolone in the hypothalamus, pedunculo pontine nucleus, and ventral striatum. Additionally, chronic pharmacological inhibition of allopregnanolone synthesis in young rats decreased allopregnanolone levels in the hypothalamus and flattened circadian activity to a level similar to that observed in aged impaired LA rats.

The present findings further detail the relationships between age-related decreases in circadian amplitude and memory impairments and extend the validation of our animal model (23) as a tool to specifically predict long-term memory impairment. Middle-aged or aged rats (LA rats) exhibiting a low amplitude of the circadian rhythm of locomotor activity had severe memory impairments in the spatial version of the water maze. In contrast, HA rats with high amplitude of the circadian rhythm of locomotor activity performed similarly to young rats. Memory impairments in LA rats likely reflected specific spatial long-term memory deficits. Thus, LA rats did not exhibit any deficits in the visual discrimination task or spatial working memory task. Increased anxiety-like behavior in certain cases can be related to cognitive impairment in adult and aged rats (39,40); however, HA and LA rats did not differ in anxiety-like behavior supporting the specificity of these memory impairments. Finally, only the circadian amplitude index was a significant predictor of spatial memory impairment, while none of the variance observed in memory performance was explained by changes in working memory, visual discrimination, locomotor performance, or age.

Measuring the circadian amplitude of activity is an index of the resistance to change of the circadian system and is one of the primary tools for defining and diagnosing sleep and circadian rhythm sleep disorders defined by the International Classification of Sleep Disorders (41,4). Long-term recordings of locomotor activity and measures of the amplitude of the sleep/wake circadian rhythm performed in the current study are indirect but reliable measures of non-REM sleep integrity (42-44). Specifically, a low circadian amplitude index reflects fragmentation of non-REM sleep (i.e., increased number of non-REM sleep episodes associated with decreased average duration of non-REM sleep episodes), consistent with results demonstrated in aged LA rats (14). Consequently, given that long-term memory, and in particular declarative memory, requires a phase of consolidation of memory during sleep (7,45-47), the memory deficits observed in the present study are likely to have resulted from an alteration of the sleep-dependent phase of memory consolidation.

Alterations of the amplitude of circadian activity were associated with modifications of neurosteroid content in specific brain regions. Compared with HA animals, LA rats exhibited a two-fold decrease of allopregnanolone levels in structures involved in sleep regulation (7), such as the hypothalamus, pedunculo pontine nucleus, and ventral striatum. In contrast, no difference was observed in regions more involved in the control of memory

consolidation, such as the hippocampus, dorsal striatum, and amygdala. These findings support the hypothesis that allopregnanolone may be involved in mediating sleep disturbances that, in turn, may lead to memory impairments. In particular, the hypothalamic decrease of allopregnanolone content observed in LA rats compared with young and HA rats may produce alterations in circadian rhythmicity, while the pedunculopontine increase of allopregnanolone observed in HA rats compared with young and LA rats may counteract age-induced alteration in sleep architecture (44,48).

The decrease of brain allopregnanolone levels observed in LA rats compared with HA rats is unlikely to be explained by a general disruption of steroidogenesis. First, the decrease of allopregnanolone was not observed in all cerebral structures analyzed. Second, this decrease was not associated with a decrease of pregnenolone, the precursor of all neurosteroids; rather, an increase in plasma level of pregnenolone was observed in LA rats. Third, concentrations of the other neurosteroids did not differ between HA and LA rats in the different structures analyzed. Finally, LA and HA rats did not differ in plasma levels of allopregnanolone, highlighting the involvement of region-specific brain mechanisms independent of peripheral steroidogenesis. Altogether, these observations suggest that specific brain region-dependent alterations in biosynthesis/degradation or storage/release of allopregnanolone are responsible for the observed decrease in allopregnanolone levels.

The link between allopregnanolone and the behavioral impairment observed in LA rats is strengthened by the observation that inhibition of allopregnanolone biosynthesis using indomethacin, a specific inhibitor of 3α -HSOR (37,65), decreased allopregnanolone levels specifically in the hypothalamus and decreased the amplitude of circadian activity in young rats. The high level of allopregnanolone in the hypothalamus and the higher sensitivity of the hypothalamus to the effect of indomethacin on allopregnanolone levels compared to the other brain regions suggest that this region represent a key structure for the synthesis of brain allopregnanolone. The causal relationship observed here between decreased allopregnanolone levels and sleep/wake pattern in young rats is consistent with our correlative results obtained with aged rats demonstrating decreased allopregnanolone levels in the hypothalamus and decreased circadian amplitude index. These results are also consistent with previous reports showing a sleep-promoting effect of allopregnanolone in rats (49,50). Allopregnanolone stimulates three key parameters of sleep-dependent memory consolidation: the propensity to fall asleep, the time spent in non-REM sleep, and the EEG power in the spindles band (12-15 Hz) (49,50). Importantly, in the context of aging, a neuroprotective role of allopregnanolone after traumatic brain injury has also been shown (51-56). Finally, further strengthening the relationships between allopregnanolone and the behavioral impairment observed in LA rats is the observation that acute administration of similar doses of indomethacin in rats impaired spatial long-term memory (57,58,59) in the water maze. Follow-up longitudinal studies will be important to determine the effect of long-term chronic allopregnanolone synthesis inhibition on neurosteroidogenesis and on the development of sleep and memory impairments during aging.

Our results conflict with other studies showing that allopregnanolone administration in young subjects impaired learning and memory (18,20,60,61). However, our data are consistent with other studies showing positive correlations between allopregnanolone levels and memory performance (62). Moreover, in female rats, cognitive improvement has been observed after allopregnanolone administration (62-64). These results further suggest that the classical pharmacological (amnesic) effect of acute administration of allopregnanolone is unrelated to the endogenous physiological role of allopregnanolone and that further studies are needed to clarify the dose range at which exogenous administration of allopregnanolone allows for the study of physiological vs. pharmacological effects.

In conclusion, this study demonstrates using correlational and causal approaches that allopregnanolone in the hypothalamus, pedunculo pontine nucleus, and ventral striatum regulates the sleep/wake cycle in young and aged rats and that alteration of basal forebrain and brainstem levels of allopregnanolone during aging may represent a key factor leading to sleep-dependent memory impairments. Allopregnanolone may play a critical role in preserving individuals from age-induced alterations in sleep and memory processes and may represent a novel target for attenuating age-related declines in memory function through improved sleep quality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GC/MS	gas chromatography/mass spectrometry
HA	high amplitude
LA	low amplitude
REM	rapid eye movement

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Figure 1. Anatomical localization of brain samples

Circles represent the exact size (scaled to the atlas) and position of each punch. The diameter of the punches ranged from 1.6 to 2 mm. The thickness of each slice was determined to maximize the specificity of each sample. AMY, amygdala; PPT, pedunculopontine nucleus; HT, hypothalamus; HPC, hippocampus; VS, ventral striatum; DS, dorsal striatum.

Figure 2. NCI GC/MS chromatogram of allopregnanolone in rat brain sample
Representative chromatogram of the relative abundance of endogenous allopregnanolone (m/z 407.3) and its internal standard, allopregnanolone-d4 (m/z 411.3).

Figure 3. Behavioral characterization of HA and LA rats. (A) Circadian amplitude index in 3-, 16-, and 22-month-old rats. (B) Determination of High Amplitude (HA) and Low Amplitude (LA) rats among the 16- and 22-month-old rat populations. The horizontal lines represent the mean of young (Y, 3-month-old) rats $\pm$ 95% confidence interval (CI). (C) Circadian amplitude index in Y, HA, and LA rats.

Figure 4.

LA rats exhibit long-term spatial memory impairment. (A) Distance to reach the platform in the water maze using distal cues for the last 2 days of place discrimination phase. (B) Distance to reach the platform in the water maze using proximal cues. (C) Distance to reach the platform in the water maze using the spatial working memory protocol. All groups showed a similar decrease in distance traveled between the first and last three trials of the last day of performance. (D) Anxiety-like behavior. The y-axis represents the loading score on the first factor extracted from the principal component analysis. This factor represents an index of anxiety-like behavior and accounted for 57% of the total variance in the open field and the elevated plus maze test. HA and LA rats exhibited a similar increase in anxiety-like behavior compared with Y rats. Data are expressed as mean $\pm$ SEM. * $p < 0.05$ vs. Y; *** $p < 0.001$ vs. Y; ### $p < 0.001$ vs. LA; \$ $p < 0.001$ vs. last three trials.

Figure 5.

LA rats exhibit lower allopregnanolone concentrations in specific cerebral structures. (A-N) Concentration of allopregnanolone and pregnenolone in the hypothalamus (A, G), pedunculo-pontine nucleus (B, H), ventral striatum (C, I), hippocampus (D, J), dorsal striatum (E, K), amygdala (F, L), and plasma (M-N). Inset in N represents the same data with an adjusted scale. Neurosteroid concentrations exhibiting a significant difference (main effect of group with ANOVA) between Y, HA, and LA are indicated with a grey background. Data are expressed as mean $\pm$ SEM fmol/mg protein or fmol/10 μ L plasma. * $p < 0.05$ vs. HA; # $p < 0.05$ vs. Y.

Figure 6. Inhibition of allopregnanolone synthesis decreases the circadian amplitude index to a level similar to LA rats

Circadian amplitude index in vehicle-treated (Control) and indomethacin-treated rats (IND 02, 2 mg/kg/day; IND 10, 10 mg/kg/day). For comparison purposes, the circadian amplitude index of HA and LA rats is represented by the two dotted lines in Fig. 1C. ** $p < 0.01$ vs. Control. Data are expressed as mean $\pm$ SEM.