Compensatory sleep response to 12 h wakefulness in young and old rats

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SIGNIFICANT CHANGES IN VARIOUS sleep parameters occur with age in mice (10, 34), rats (14, 16, 22, 32, 35), cats (4, 5), monkeys (18), and humans (for review, see Refs. 1 and 20). These changes include sleep fragmentation, increased wake time, and decreases in the length of sleep bouts, amplitude of the diurnal rhythm of sleep, and rapid eye movement (REM) sleep (for review, see Ref. 1). There is also a pronounced decline in the day-night rhythm of sleep-wakefulness (6, 14), suggesting that the SCN is affected by age. Peak neuronal discharge from the SCN of old (23–27 mo) rats is reduced and more erratic compared with young rats (23), and the SCN has been found to be smaller in older humans (80 years old; Ref. 28). On the other hand, recent findings in healthy older subjects who have no sleep complaints indicate that they do not have any change in the period of the temperature rhythm (8). A longitudinal study in hamsters also recently demonstrated stability in the period of wheel running activity with age (9). These findings raise the possibility that there is no change in the pacemaking ability of the SCN with age.

Sleep is also regulated by a homeostatic component that builds gradually with wakefulness and then dissipates with sleep (3). The shortened sleep bouts in the elderly could result from an age-related decline in mechanisms that build sleep drive during wakefulness. The best method used to gauge the sleep drive is to first prolong wakefulness and then examine the increase in the ensuing sleep (3). In older humans, the compensatory sleep response to prolonged wakefulness is generally less intense and short lived (2, 7, 33), indicating a decline in sleep drive. Surprisingly, the effect of prolonged wakefulness on subsequent sleep has not been studied in old animals. The availability of such data could facilitate investigations into the effects of aging on the underlying neuronal mechanisms regulating sleep.

The present study examined the compensatory sleep response to 12 h prolonged wakefulness in two strains of rats, the Sprague-Dawley and F344 rats. The results indicate that in both strains, old rats demonstrated significantly lower levels of slow-wave sleep (SWS) compared with young rats in response to prolonged wakefulness. The recovery of REM sleep was not...
affected by age. To identify one possible neuronal substrate contributing to the reduced sleep pressure in old rats, we counted the numbers of c-Fos immunoreactive (c-Fos-ir) cells in the ventral lateral preoptic (VLPO) area. Recent studies demonstrated that neurons in the VLPO are important in generating sleep (for review, see Ref. 27) based on neurotransmitter identity (25), projections of VLPO neurons to arousal centers (25), and selective activity of VLPO neurons during sleep (26, 30). We found that the number of c-Fos-ir neurons in VLPO was not different between young and old rats. This suggests that an age-related decline in sleep regulatory mechanisms other than loss of sleep-active VLPO neurons contributes to the decreased compensatory sleep response in old rats.

METHODS

Young (2 mo) and old (20 mo) Sprague-Dawley (n = 19) or F344 (n = 16) rats were obtained from the vendor (Sprague-Dawley; Camm Research Labs; F344: National Institutes of Aging) and allowed to recover in our vivarium for 1 mo from the stress associated with transport and delivery. Thereafter, the rats were implanted under anesthesia [cocktail of acepromazine (0.75 mg/kg), xylazine (2.5 mg/kg), and ketamine (22 mg/kg) administered intramuscularly] with sleep recording electrodes. Four stainless steel screw electrodes were positioned in the skull to sit on the surface of the cortex and were used to record the EEG. Two miniature screws were inserted 2 mm on either side of the sagittal sinus and 3 mm anterior to bregma (frontal cortex). The other two screws were located 3 mm on either side of the sagittal sinus and 6 mm behind bregma (occipital cortex). The EEG was recorded from two contralateral screws (frontal-occipital). To record muscle activity [electromyogram (EMG)], two flexible multistranded wires were inserted in the nuchal muscles. The six electrodes were placed in an amphenol plug and secured onto the skull using dental cement. The animals were housed singly in Plexiglas cages with wood shavings; food and water were available ad libitum. The temperature in the sleep recording room was 25°C and a 12:12-h light-dark cycle (0700–1900 lights on; 100 lx) was maintained. A physical examination at the time the electrodes were implanted indicated that the rats did not have tumors. However, at the conclusion of the experiment, two old F344 rats were found to have pituitary masses and were excluded from the study. The sample size for F344 rats (n = 16) does not include these two rats.

Two weeks after recovery from surgery, the animals were connected to lightweight recording cables and adapted for 3 days. After the adaptation period, the EEG and EMG were recorded for a consecutive 48-h period. The rats were kept awake for 12 h from 0700 (lights on) to 1900 (lights off) and then allowed to sleep undisturbed, and sleep recordings were obtained for 24 h. The rats were kept awake by lightly tapping the cage and periodically introducing crumpled pieces of paper into the cage. EEG recordings were obtained throughout the enforced wakefulness and postwakefulness periods. The EEG and EMG signals were recorded on a Grass model 9 polygraph and also recorded onto a jaz disk using an analog-to-digital board (National Instruments).

One week after the end of the 12-h prolonged wakefulness protocol, the Sprague-Dawley animals were kept awake for 6 h (1300–1900). Some animals were killed immediately at the end of the prolonged wakefulness, whereas some animals were allowed 1 h of sleep and then killed. This protocol was used to facilitate detection of VLPO neurons in response to sleep. The rats were deeply anesthetized with Nembutal and perfused transcardially with 0.9% saline (50 ml) followed by 4% paraformaldehyde in 0.1 M PBS (200 ml). The brains were placed in 20% sucrose (0.1 M PBS) and allowed to equilibrate. This group of animals was used to identify c-Fos-ir labeled cells in the VLPO.

Analysis of sleep data. Contralateral frontal-occipital EEG screw electrodes were used for EEG acquisition. The EEG data were filtered at 70 Hz (low-pass filter) and 0.3 Hz (high-pass filter) using a Grass electromyograph and continuously sampled at 128 Hz. The 48-h EEG and EMG recordings were scored manually on a computer (Celus software, M. Opp) in 12-s epochs for awake, SWS, and REM sleep by staff (J. Thakkar, Sprague-Dawley rats; S. Shironmani and U. Upadhaya, F344 rats) blind to the age and strain of the animals. Wakefulness was identified by the presence of desynchronized EEG and high EMG activity. SWS consisted of high-amplitude slow waves together with a low EMG tone relative to waking. REM sleep was identified by the presence of regular theta activity coupled with low EMG relative to SWS. The amount of time spent in wakefulness, SWS, and REM was determined for each hour for the 48 h and then averaged to yield 24 h. To determine whether there was a change in the amplitude of the diurnal rhythm of sleep, the ratio of sleep during the light-on period versus the light-off period was calculated (Table 1). Delta power (0.5–4 Hz) was calculated using the Celus software system. After the EEG data were scored, the code was broken to reveal the identity of each rat. ANOVA and t-tests with Bonferroni correction (where appropriate) were used to compare changes in sleep parameters. Statistical significance was evaluated at the P < 0.05 level.

Immunohistochemistry and cell counting. The brains were cut (frozen sections, 40 µm, coronal), and tissue sections were incubated overnight at room temperature in the c-Fos primary antibody as previously described (26; ABS, Oncogene Science, 1:150,000). The next day, the following protocol was used: 1) two washes in 0.1 M PBS; 2) 1-h incubation in secondary antibody (1:250); 3) two washes; 4) 1-h incubation in avidin-biotin complex; 5) two washes; 6) 5 min in 3',3'-diaminobenzidine-nickel chloride-hydrogen peroxide solution. The c-Fos-ir neurons were counted as per methods described previously (26). Briefly, the VLPO was identified in the coronal plane beginning at the rostral pole of the supraop-

| Table 1. Changes in light-dark ratios of different states in young and old rats |
|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                      | F344             | Sprague-Dawley   |                  |                  |                  |                  |                  |                  |
|                      | W                | SWS              | REM              | W                | SWS              | REM              |                  |                  |
| Old-BL               | 0.479 ± 0.03     | 2.35 ± 0.16      | 2.43 ± 0.57      | 0.496 ± 0.047    | 1.61 ± 0.1*      | 2.15 ± 0.37      |                  |                  |
| Young-BL             | 0.463 ± 0.05     | 2.94 ± 0.51      | 3.12 ± 1.22      | 0.42 ± 0.039     | 2.08 ± 0.19      | 2.72 ± 0.45      |                  |                  |

Values (±SE) represent ratio of wake (W), slow-wave sleep (SWS), or rapid eye movement (REM) percent during light-on period versus its corresponding value during light-off period. Old Sprague-Dawley rats demonstrated a significant reduction in amplitude in diurnal rhythm of SWS (*P < 0.04, t = 2.551). BL, baseline.
tic nucleus and extending rostrally until the decussation of the anterior commissure (bregma − 0.26). c-Fos-ir cells within an area 1 mm from the midline to the edge of the olfactory tubercle and extending 0.3 mm dorsally were counted. Counts were obtained bilaterally from at least three sections (in a 1-in-4 series), and the numbers represent the average counts from one section from one side. Photomicrographs of c-Fos-ir cells in VLPO were obtained using a Kodak 420 digital camera and Adobe Photoshop and printed on a Kodak 8650 printer.

RESULTS

Basal levels of sleep parameters. In the F344 rats (8 young, 8 old), there were no differences between the two age groups in baseline levels of wakefulness or SWS (see Fig. 1). However, old F344 rats had significantly less REM sleep during the light-on period compared with the young F344 rats (t = 2.84, degrees of freedom (df) = 14, P < 0.01). REM sleep expressed as a percentage of total sleep time was also significantly less in the light-on period in the F344 rats (t = 2.33, df = 14, P < 0.04). The decrease in REM sleep was a result of a significant decrease in the length of REM sleep bouts (P < 0.05) and not a result of a decrease in the number of REM sleep bouts (see Table 2). The decreased length of the REM sleep bouts occurred during both the light-on and light-off periods (see Table 2 for comparison). The reduction in REM sleep during the light-on period (Fig. 1) would suggest a reduction in amplitude of the diurnal rhythm of REM sleep in F344 rats. However, the light-dark ratio of REM sleep was not significant (Table 1). Old F344 rats also had significantly shorter SWS bouts compared with the young rats, but only during the light-off period (P < 0.05).

In the Sprague-Dawley rats there were no significant differences in wakefulness, SWS, or REM sleep (Fig. 1) between young (n = 4) and old (n = 4) rats. Table 3 summarizes the average number and length of wake, SWS, and REM sleep bouts during baseline and recovery sleep in young and old rats. There were no significant differences between young and old rats in number or duration of wake, SWS, or REM sleep bouts during the baseline period (either light-on or light-off period). There was a significant reduction in the diurnal rhythm of SWS in the old compared with young Sprague-Dawley rats (t = 2.551, df = 6, P < 0.04; Table 1). Figure 2 summarizes the delta power in young and old rats. Old F344 rats demonstrated a significant decrease in delta power (0.5–4 Hz) compared with young F344 rats across the 24-h recording period (F (1,15) = 26.54, P < 0.002). In the old Sprague-Dawley rats, delta power across the 24 h was not statistically significant compared with young rats. However, in both young and old rats there was a gradual reduction in delta power across the light-on period, a finding that is consistent with published reports (see Ref. 3).

Effects of prolonged wakefulness on sleep parameters. After a 48-h baseline sleep record was obtained, the rats were kept awake for 12 h (0700–1900, normal sleep time for rats) and then allowed to sleep (1900, lights off). The EEG was recorded throughout the 12-h period of wakefulness and during the subsequent 24 h. Figures 3 and 4 summarize the difference in sleep from basal levels during the first and second 12-h recovery sleep periods, respectively. In the first 12 h of recovery sleep, the young F344 rats had a significant increase in total sleep time (TST; t = 4.29, P < 0.005) and SWS (t = 4.09, P < 0.001) compared with the old rats. However, the old F344 rats had more REM sleep compared with the young rats (t = 5.15, P < 0.002).

Similar results were obtained in the Sprague-Dawley rats, where the significant difference in TST between young and old rats [117 vs. 53% (t = 2.77, P < 0.03)] could be explained by a significant increase in SWS in younger rats compared with old rats (t = 3.0, P < 0.02). Young and old rats had similar increases in REM sleep, however. Thus, for both F344 and Sprague-Dawley rats, the young rats slept more because they had significantly more SWS.

During the first 12 h of recovery sleep (light-off period), young and old rats in both strains had a

Table 2. Average number of transitions to SWS, REM, or W and duration of W, SWS, or REM in young and old F344 rats

<table>
<thead>
<tr>
<th></th>
<th>Average Number of Transitions</th>
<th>Average Duration of Bouts, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W-SWS</td>
<td>SWS-REM</td>
</tr>
<tr>
<td>Light-off period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old-BL</td>
<td>51.75±3.8</td>
<td>34.13±4.4</td>
</tr>
<tr>
<td>Old-RS</td>
<td>90.5±4.6*</td>
<td>91.25±4.7*</td>
</tr>
<tr>
<td>Young-BL</td>
<td>46.63±4.6</td>
<td>27.63±2.8</td>
</tr>
<tr>
<td>Young-RS</td>
<td>87.50±6.8*</td>
<td>63.75±5.4*</td>
</tr>
<tr>
<td>Light-on period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old-BL</td>
<td>95.6±5.4</td>
<td>55.8±3.1</td>
</tr>
<tr>
<td>Old-RS</td>
<td>77.80±6.6</td>
<td>43.25±6.18</td>
</tr>
<tr>
<td>Young-BL</td>
<td>95.25±5.9</td>
<td>47.33±5.0</td>
</tr>
<tr>
<td>Young-RS</td>
<td>85.5±5.4</td>
<td>36.25±2.8</td>
</tr>
</tbody>
</table>

Numbers represent averages (± SE; n = 8 old and 8 young rats) derived for BL and recovery sleep (RS). Old rats had significantly shorter REM sleep bouts during light-on and light-off periods and shorter SWS bouts during light-off period compared with young rats. In response to 12 h prolonged W, during first 12 h of recovery sleep (light-off period), both old and young rats had shorter wake bouts, more entries into SWS, and more frequent entries into REM sleep. In old rats, length of REM sleep bouts increased significantly. Increase in SWS in young rats was influenced by more frequent entries into SWS compared with old rats. *Significance (P < 0.05) for each group compared with its respective value during baseline; †significant (P < 0.05) difference between young and old rats during baseline.
significant decrease in the length of the wake bouts and increases in entries into both SWS and REM sleep (see Tables 2 and 3 for specific comparisons). In young rats, the increase in SWS resulted from more frequent entries into SWS (young F344 rats) or from an increased length of SWS bouts (Sprague-Dawley). For example, from Table 3 it can be seen that during the first 12 h of recovery sleep, the older rats woke up (transitions from SWS to wake) as often as during baseline light-off period, but the younger rats were almost half as likely to wake up compared with their baseline light-off period (P < 0.05). Thus old rats woke up more often and had shorter duration SWS bouts. The short sleep cycle in the old rats is maintained even after prolonged wakefulness, suggesting an age-related loss of ability to maintain sleep.

Figure 5 depicts changes in delta power after prolonged wakefulness in young and old rats. There was a significant increase in relative delta power in both young and old rats during the first 12 h of recovery.

Table 3. Average number of transitions to SWS, REM, or W and duration of W, SWS, or REM in young and old Sprague-Dawley rats

<table>
<thead>
<tr>
<th></th>
<th>Average Number of Transitions</th>
<th>Average Duration of Bouts, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W-SWS</td>
<td>SWS-REM</td>
</tr>
<tr>
<td><strong>Light-off period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old-BL</td>
<td>82.50 ± 5.2</td>
<td>48.63 ± 6.1</td>
</tr>
<tr>
<td>Old-RS</td>
<td>111.67 ± 5.1*</td>
<td>84.00 ± 2.4*</td>
</tr>
<tr>
<td>Young-BL</td>
<td>75.88 ± 3.7</td>
<td>25.75 ± 2.0</td>
</tr>
<tr>
<td>Young-RS</td>
<td>92.00 ± 5.67*</td>
<td>63.67 ± 2.2*</td>
</tr>
<tr>
<td><strong>Light-on period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old-BL</td>
<td>73.50 ± 4.2</td>
<td>48.00 ± 5.1</td>
</tr>
<tr>
<td>Old-RS</td>
<td>104.0 ± 11.3</td>
<td>65.33 ± 10.6</td>
</tr>
<tr>
<td>Young-BL</td>
<td>75.75 ± 6.0</td>
<td>39.63 ± 2.5</td>
</tr>
<tr>
<td>Young-RS</td>
<td>106.67 ± 2.7</td>
<td>60.33 ± 0.6</td>
</tr>
</tbody>
</table>

Numbers represent averages (±SE; n = 4 young and 4 old rats) during BL and RS. There were no significant differences between old and young rats during baseline in light-on or light-off periods. After 12 h prolonged wakefulness, both old and young rats had more entries into SWS, but young rats stayed in SWS for a longer time. *Significance (P < 0.05) for each age group compared with its respective baseline value.
sleep ($t = 3.84$, $P < 0.002$). During the subsequent 12 h (light-on period), delta power declined significantly ($t = 3.57$, $P < 0.003$).

Numbers of c-Fos-ir labeled cells in young versus old rats in the VLPO area. The numbers of c-Fos-ir labeled cells in the VLPO were not different between young and old rats, either after wakefulness or after sleep (Fig. 6). Young and old rats that were killed immediately at the end of 6 h prolonged wakefulness had few c-Fos-ir labeled cells in VLPO (Fig. 6A). In response to sleep, the numbers of c-Fos-ir labeled cells increased significantly in both young and old rats (mean number of c-Fos-ir cells in VLPO in awake rats $7.45 \pm 3.05$, $n = 7$; mean number c-Fos-ir cells in VLPO in asleep rats $18.85 \pm 0.30$, $n = 12$; independent $t$-test with Bonferroni adjustment $4.135$; $df = 17$, $P < 0.001$). However, both young and old rats demonstrated similar numbers of c-Fos-ir labeled cells in VLPO. The amount of sleep obtained by the two age groups during the 1 h before death was similar (Fig. 6B). However, old rats were unable to maintain sleep, especially SWS, over the course of a 12-h period (see Fig. 3). Figure 7 depicts c-Fos-ir labeled neurons in the VLPO of young and old rats.

**DISCUSSION**

This study found that after prolonged wakefulness, old rats slept less compared with young rats. This would suggest an age-related decline in sleep drive in response to wakefulness. To determine whether neuronal loss could be a contributing factor, the number of sleep-active neurons, as determined by c-Fos immunoreactivity, in the VLPO were counted and found to be similar in young and old rats. The difference in TST between young and old rats was due to older rats having less SWS in response to prolonged wakefulness. Both young and old rats had robust increases in REM sleep. This effect was found in both the F344 and Sprague-Dawley rats, suggesting that in both strains of rats, aging significantly decreases homeostatic mechanisms regulating SWS.

Basal sleep parameters. Age-related changes in sleep parameters have been examined previously in Sprague-Dawley (35), F344 (16, 22), Wistar (32), and Long-Evans (14) rats. The age of young rats used in those studies were 2 (35), 4 (22, 32), and 6–8 mo (14). In the previous studies, the age of the old rats ranged from 22 to 27 mo (14, 16, 22, 32, 35). In previous studies, as in ours, only male rats were studied.

Zepelin et al. (35), monitored Sprague-Dawley rats and did not observe any discernible differences in sleep-wake states or delta power between young and old rats. Li and Satinoff (14) recorded sleep from Long-Evans rats, and, although some of their old rats had severe loss of temperature rhythms, the time spent in sleep or wakefulness was not affected by age. However, they did observe that the amplitude of the light-
dark rhythm of sleep was attenuated in old rats (14). In the present study, we also did not find any differences in the basal levels of sleep or delta power in Sprague-Dawley rats, and, as previously reported (14), the diurnal rhythm of SWS was less in old rats.

Two studies (16, 22) monitored sleep in young and old F344 rats (obtained from National Institute of Aging, which was also our source of rats). Mendelson and Bergmann (16) did not find any changes in wakefulness, SWS, or REM sleep in old rats, whereas Rosenberg et al. (22), found that old F344 rats were awake more and had less REM sleep. They did not find any changes in SWS. Van Gool and Mirmiran (32) recorded Wistar rats and found decreases in wakefulness and REM sleep. In our study, we did not find any difference in wakefulness or SWS in the F344 rats, but like Rosenberg et al. (22) and Van Gool and Mirmiran (32), we found that old F344 rats had less REM sleep. Recently, Mendelson and Bergmann (16) reported a decrease in high-voltage SWS (which we deduce to be delta) in 24-mo-old F344 rats, and this is similar to our finding.

Overall, our results concur with previous studies in that some basal sleep variables are affected by age in rats, but this depends on the strain of the rat. In the sleep study in old mice (10), the effects of strain on sleep were also observed. Although, a number of studies have examined age-related changes in sleep in animals, we
are unaware of a published study that has investigated whether the compensatory sleep response to prolonged wakefulness is affected by age.

Compensatory sleep response to prolonged wakefulness. Significant differences in sleep between young and old rats were found in response to 12 h prolonged wakefulness. Young rats had more rebound SWS compared with old rats. In the young rats, the length of the SWS bouts during the first 12 h of recovery sleep increased compared with baseline light-off period. Moreover, the younger rats did not wake up as frequently during the first 12 h of recovery sleep. Both young and old rats had similar increases in REM sleep, suggesting that homeostatic mechanisms regulating REM sleep are not impaired with age. Old F344 rats especially were able to mount a vigorous REM sleep rebound. This suggests that in both F344 and Sprague-Dawley strains, homeostatic responses regulating REM sleep are not affected by age.

These findings are similar to elderly humans where compensatory sleep response to prolonged wakefulness is generally less intense and short lived (2, 7, 33).

Delta power. SWS is identified by the presence of high-voltage slow waves (also referred to as delta activity) that occur in the 0.3- to 4-Hz range. Considerable research in both humans and rodents has shown that delta activity builds gradually with wakefulness, is highest at the start of the sleep period, and declines progressively with sleep (for review, see Ref. 3). This waxing and waning of delta activity is considered to represent an overt expression of an endogenous homeostat that regulates sleep drive (3). In the present study (see Fig. 2), we also found a buildup of delta activity with wakefulness and its decline with sleep. Thus our findings of delta activity are consistent with published reports (summarized in Ref. 3).

There is a very pronounced decline in delta activity in older humans (11), monkeys (18), and cats (4, 5). The results of the present study and that of a recent report (16) indicate that old F344 rats have significantly less delta activity compared with young rats. However, SWS time was not significantly different between age groups. Thus, in the F344 rats, there was dissociation between delta activity and SWS time; delta power was reduced without a corresponding decline in SWS time. In response to 12 h prolonged wakefulness, both delta power and SWS time increased in the young and old rats.

Such an age-related dissociation between delta power and sleep time has been reported recently by Frank et al. (13), who found that in neonatal rats, the development of delta activity lags behind sleep time. Specifically, in response to 3 h prolonged wakefulness, neonatal rats younger than postnatal day 24 did not show an increase in delta activity but did show an increase in sleep time. The Frank et al. findings, taken together with the present findings, suggest that changes in sleep time might represent a good indicator of age-related changes in sleep regulation.

Number of c-Fos-ir sleep-active cells in VLPO. Because the old rats had less SWS rebound, the VLPO area was examined to determine whether there was a reduction in sleep-active cells in old rats. The VLPO was originally identified using c-Fos as a neuroanatomical marker of neuronal activity (26). Neurons in this region are GABAergic and project monosynaptically to major arousal neurons (25, 26). The VLPO cells are hypothesized to inhibit wake-active cells to induce sleep (26). Electrophysiology studies have now identified that cells in this region have their highest discharge during sleep relative to wakefulness (30). Such electrophysiological evidence provides support for the finding of c-Fos-ir neurons in response to sleep in the VLPO. Electrophysiology studies have found sleep-active cells in the preoptic area and the basal forebrain (29). However, in the VLPO there is a greater concentration of electrophysiological sleep-active cells compared with other regions (30).

In the present study, the numbers of c-Fos-ir labeled cells were not different between young and old rats.
after sleep. Thus, in old rats, there does not appear to be a decline in the number of sleep-active cells in the VLPO, as identified by c-Fos-ir labeling. Additional experiments that use stereological measures and count the number of neurotransmitter-containing cells in the VLPO would be necessary to more conclusively evaluate the extent of cell loss in old rats. Such studies could count the number of GABA- and/or galanin-labeled cells in VLPO. Both GABA and galanin have been found to identify cells in the VLPO (25), but c-Fos-ir labeling indicates the activation of cells during sleep (26).

Because old rats had similar numbers of c-Fos-ir labeled sleep-active cells in VLPO as young rats, we suggest that at least one neuronal substrate for generating sleep, as defined by the number of c-Fos-ir cells in VLPO, does not decline with age. This finding is consistent with emerging data indicating that neuronal loss is not an inevitable consequence of aging (17). For example, the number of hippocampal neurons is similar between young and old rats, although old rats have severe memory impairments (21). In our study, old rats showed a reduced compensatory sleep response to prolonged wakefulness. However, there was no difference in the numbers of sleep-active cells in the VLPO.

Although we cannot rule out that sleep-active neurons elsewhere in the preoptic area and adjacent basal forebrain do not decrease with age, our data suggest that a reduction in other functional elements of the circuitry contribute to the decline in sleep with aging. This circuitry is able to generate sleep, because young and old rats had similar increases in sleep during the 1-h postwakfulness period (Fig. 6B). However, old rats failed to maintain a sustained amount of sleep as evidenced by a decrease in the overall amounts of sleep during the 12-h period (see Fig. 3). The inability of old rats to maintain a sustained amount of sleep suggests a decline in homeostatic mechanisms regulating SWS.

This mechanism could include endogenous factors, such as adenosine (19, 31) or prostaglandins (15), that are believed to regulate sleep. Prostaglandins, such as PGD₂, have been found to induce c-Fos-ir in neurons relevant for sleep (24). Because the present study found that there is no loss of VLPO neuronal activation, there is an impetus for investigating whether a decline in putative somnogens, receptors, and/or a coupling between extracellular factors (neurotransmitters, sleep factors) and intracellular cascades could contribute to the decreased sleep observed with age (27).

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