Control and Function of the Homeostatic Sleep Response by Adenosine A$_1$ Receptors

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During sleep, the mammalian CNS undergoes widespread, synchronized slow-wave activity (SWA) that directly varies with previous waking duration (Borbély, 1982; Dijk et al., 1990). When sleep is restricted, an enhanced SWA response follows in the next sleep period. The enhancement of SWA is associated with improved cognitive performance (Huber et al., 2004), but it is unclear whether the SWA is enhanced or whether SWA is needed to maintain normal cognitive performance. A conditional, CNS knock-out of the adenosine receptor, AdoA$_1$R, gene, shows selective attenuation of the SWA rebound response to restricted sleep, but sleep duration is not affected. During sleep restriction, wild phenotype animals express a rebound SWA response and maintain cognitive performance in a working memory task. However, the knock-out animals not only show a reduced rebound SWA response but they also fail to maintain normal cognitive function, although this function is normal when sleep is not restricted. Thus, AdoA$_1$R activation is needed for normal rebound SWA, and when the SWA rebound is reduced, there is a failure to maintain working memory function, suggesting a functional role for SWA homeostasis.

Key words: sleep; delta; adenosine; working memory; hippocampal function; memory; Cre-transgenic; metabolism

Introduction

The need for recovery of lost sleep, the homeostatic sleep response, is considered one of the universal characteristics of sleep (Mignot, 2008). One commonly used measure of this response is slow-wave activity (SWA) (Dijk et al., 1990). SWA is measured from the electroencephalogram and results from synchronized, neuronal membrane potential fluctuations of populations of cortical and thalamocortical neurons, with a dominant frequency component in the range of 0.5–4.5 Hz (Amzica and Steriade, 1998). Slow-wave activity is present across states but is predominately in the EEG during slow-wave sleep (SWS), also called non-rapid eye movement sleep, and in this sense, SWS may be considered permissive to the expression of SWA.

The magnitude of SWA is directly correlated with the time spent awake before the SWS episode (Franken et al., 2001) and may be locally increased in a use-dependent manner (Huber et al., 2004; Vyazovskiy et al., 2004). Thus, SWA is considered a marker for and is thought to be functionally involved in sleep/waking homeostasis (Daan et al., 1984).

Adenosine has been hypothesized to be involved in sleep/waking homeostasis by modulation of SWA. Converging evidence suggests that this SWA modulation results from A$_1$ adenosine receptor (AdoA$_1$R) activation. Adenosine, acting on the AdoA$_1$R, has been shown to decrease cholinergic neuronal activity and to facilitate, at the single-cell level, slow oscillations (Pape, 1992; Rainnie et al., 1994; Benington et al., 1995; Porsky-Heiskanen et al., 1997). Pharmacological blockade of adenosine A$_1$ and A$_2$ receptors by caffeine results in increased arousal (and decreased sleep) in mammals (Fredholm et al., 2005). In rodents, the increased arousal effect may, in large part, be secondary to increased locomotor activity (Lindskog et al., 2002), mediated by the A$_2$ AdoR signaling system in the striatum that is, in itself, arousing. However, an AdoA$_1$R-mediated action cannot be ruled out despite the evidence that the stimulatory action of caffeine is less marked acting by AdoA$_1$Rs (Huang et al., 2005). Furthermore, the SWA-promoting actions of increased endogenous or exogenous adenosine in the cholinergic nuclei (Porsky-Heiskanen et al., 1997; Portas et al., 1997) seems most likely to be mediated by AdoA$_1$R, because AdoA$_2$Rs have no electrophysiological activity in the cholinergic nuclei, in contrast to AdoA$_1$Rs (Arrigoni et al., 2001). Thus, the physiological role of AdoA$_1$Rs to increase SWA remains to be resolved.

We have used conditional AdoA$_1$R knock-out mice, using a calcium/calmodulin-dependent protein kinase II (CaMKII)-promoting Cre transgene, to selectively reduce rebound SWA in response to moderate sleep restriction, as a means of assessing mechanisms responsible for this classic homeostatic sleep response. The conditional knock-out approach was justified over either the constitutive knock-out or the adenoassociated virus (AAV)-mediated Cre induction approaches for the following reasons. First, the constitutive deletion of the AdoA$_1$R gene showed a minimal reduction of SWA in mice during the early part of the circadian day (at lights on) when sleep pressure is most likely to be the greatest (Stenberg et al., 2003), raising the possibility of developmental compensatory effects. These potential effects would be less of a factor with a conditional gene knock-out (Tsien...
et al., 1996). Second, although localized increases in adenosine are sufficient to increase SWA (Porkka-Heiskanen et al., 1997; Portas et al., 1997), this may require activation of presynaptic AdoA1R (Arrigoni et al., 2001, 2005; Brambilla et al., 2005). A localized AAV-Cre mediated AdoA1R gene deletion will only affect the neurons within the region of transfection, leaving presynaptic AdoA1Rs on terminals originating from neurons outside the transfected region unaffected.

Evidence is provided here suggesting that the normal rebound increase in SWA in response to sleep restriction is modulated by AdoA1Rs and that this modulation is associated with normal working memory performance under sleep restriction conditions.

**Materials and Methods**

**Conditional gene deletion of AdoA1R.** A targeted gene insertion, using a C57BL/6 mouse and embryonic stem cells from 129SvJ strain, was made with loxP sites flanking the AdoA1R gene (Scammell et al., 2003). AdoA1R gene deletion was induced by crossing these mice with another strain (T50 line) containing a transgene expressing the recombinase, Cre, under the control of a CaMKII promoter (Tsien et al., 1996). The homozygous “floxed” AdoA1R strain (AdoA1Rf/f) has a wild-type expression pattern for AdoA1R message (Fig. 1, middle column). The AdoA1Rf/f strain (homozygous for the floxed allele, AdoA1Rf, and positive for the CaMKII-Cre transgene) shows a loss of AdoA1R expression, and this loss was restricted to regions that were indicated by β-galactosidase expression in ROSA26 reporter mice (Tsien et al., 1996) crossed with mice containing the CaMKII-Cre transgene (Fig. 1).

The expression pattern we observed has been previously reported (Monteggia et al., 2007), using similar histological techniques as used in our study. Cre expression was not observed in putative interneurons or in the inhibitory nucleus reticularis of the thalamus. At high-power magnification, β-galactosidase staining was clearly apparent in the nuclei of all the thalamocortical neurons (data not shown), despite its apparent absence of expression in the thalamus in low-power micrographs (Fig. 1, left column). β-Galactosidase expression was concentrated in the nuclei of cells in the ROsa26 mouse, and this appeared especially to be the case for the thalamic neurons.

We used standard autoradiographic techniques to image 35S-labeled mRNA probe for the AdoA1R transcript as described previously (Scammell et al., 2003). The homozygous “floxed” AdoA1R strain (AdoA1Rf/f) has a wild-type expression pattern for AdoA1R message (Fig. 1, middle column). The AdoA1Rf/f strain (homozygous for the floxed allele, AdoA1Rf, and positive for the CaMKII-Cre transgene) shows a loss of AdoA1R expression, and this loss was restricted to regions that were positive for both Cre and AdoA1R expression (Fig. 1, right column).

Quantitative densitometry indicates that the AdoA1R message is reduced in the AdoA1Rf/f strain by >80% in sleep-relevant areas that include the following: (1) in the brainstem, the dorsal pontine tegumentum; (2) in the diencephalon, the thalamus, with the exception of the inhibitory nucleus reticularis, and the hypothalamus; (3) in the forebrain, the parietal neocortex. Using the identical AdoA1Rf/f strain, we have previously shown that, wherever Cre is expressed, there is an associated loss of AdoA1R-dependent electrophysiological responses (Scammell et al., 2003; Arrigoni et al., 2005).
Sleep recording was complete, data were copied to CD and scored off-line using Rodent Sleep Scorer (Astro-Med). Epochs were scored in 10–15 s and assigned into one of three sleep/waking states (see Fig. 3). Waking consists of low-amplitude, high-frequency EEG and high EMG activity; SWS consists of high-amplitude, low-frequency EEG with little EMG modulation; and REM consists of low-amplitude, desynchronized EEG with occasional muscle twitches on a background of low EMG activity. Rodent Sleep Scorer (Astro-Med) also performed a fast Fourier transform analysis with a Hamming window function for each epoch. Delta power from 3.0 to 4.5 Hz was defined as slow-wave activity and averaged across all epochs and across all SWS sleep epochs for statistical comparisons across conditions. This delta power range was chosen based on the findings of Maret et al. (2005) of an altered SWA power frequency band distribution observed in mice with a mutated retinoic acid receptor β gene during SWS primarily in the 3.0–4.5 Hz range.

Working memory; eight-arm radial maze testing. A standard eight-arm radial maze protocol was used to assess working memory. The mice used for cognitive testing were not tethered to the recording system, but were otherwise treated in a similar manner to the recorded animals. All animals were weighed daily, maintained at or above 85% of their ad libitum feeding weight, and were housed on the treadmill (not moving) from training onward. The radial maze is constructed of clear plastic and placed on a table surface with visual cues from the room readily visible.

The task involved obtaining rewards that were hidden from sight in small cups at the end of each arm. Over 2 weeks of training (two trials per day), all the animals learned to visit each of the eight arms only once and never made mistakes (average, less than two mistakes per trial). These observations are similar to other assessments of hippocampal-independent working memory (Winocur, 1982) in mice using a similar radial maze protocol (Schmitt et al., 2003). Control trials in trained animals using unbaited cups confirmed that olfactory cues obtained from the reward itself were not needed to locate the correct cup.

Mice had two training sessions per day, a morning session at 8:00 A.M. and an afternoon session at 2:00 P.M. At the beginning of each training trial, eight arms were baited with a small piece of chocolate (Hershey’s Hugs; The Hershey Company). Mice were placed into the central hub of the maze and allowed to enter any arm. A revisit error was scored if a mouse traversed more than one-third of an arm from which they had already retrieved the chocolate. Mice were removed from the maze after successfully retrieving all eight chocolate pieces or after a maximum of 10 min. Training continued until animals reached asymptotic performance for 3 d, after which animals began 2 d of testing, followed by 3 d of recall. Testing procedures were the same as training, with the exception that the treadmill was set to the 6 h cycle (4 h on; 2 h off). Recall was the same as training (TM off).

Elevated maze; paired associates testing. The protocol for the paired associates task was the same as what we used previously (Rajji et al., 2006). Briefly, mice were first shaped for 10 d to dig into a sand-filled cup for a chocolate piece buried in the sand. Animals who failed to retrieve the chocolate were removed from the maze after successfully retrieving all eight chocolate pieces or after a maximum of 10 min. Training continued until animals reached asymptotic performance for 3 d, after which animals began 2 d of testing, followed by 3 d of recall. Testing procedures were the same as training, with the exception that the treadmill was set to the 6 h cycle (4 h on; 2 h off). Recall was the same as training (TM off).

Statistical analyses were performed using GraphPad Prism (GraphPad Software). Group comparisons of sleep time and SWA were made using a Mann–Whitney U test. Data are expressed as mean ± SEM. For baseline (undisturbed time) sleep/waking time analyses, percentage time in waking, SWS, and REM sleep was calculated.
from four 2 h blocks (matched to the TM-off times: 12:00–2:00 P.M., 6:00–8:00 P.M., 12:00–2:00 A.M., 6:00–8:00 A.M.). Animals showing >90% waking in any baseline 2 hour block were eliminated from additional analysis. Time in SWS over the 24 h baseline period was calculated for a subset of mice for comparisons of daily SWS time. For SWA analyses, SWA was averaged in 1 h bins for baseline and both sleep restriction days. For the eight-arm radial maze, the number of revisit errors and latency to complete the task were averaged for two sessions, one beginning after 18 h of sleep restriction and the other 24 h later. Comparisons were made between the average performance on the last two training days, sleep restriction days, and recovery day, along with weight on each of these days. A Wilcoxon matched-pairs test was used to compare error rate between baseline and probe conditions within each group. A Mann–Whitney U test was used to compare error rate, latency, and weight between groups. For the paired associates task, percentage correct on the new context trials during testing was calculated. A two-way ANOVA was used to compare genotype (AdoA1Rf/f and AdoA1Rf/f mutants) and time (trial 1 and trial 5).

Results

Spontaneous waking, SWS, and slow-wave activity

The expression of baseline sleep/waking states were not different between genotypes (Fig. 3). The AdoA1Rf/f mice had a similar percentage time in waking (n = 5; 47.2 ± 9.2%) (Fig. 3A), SWS (43.9 ± 7.9%) (Fig. 3A), and REM sleep (8.2 ± 2.5%) (data not shown) compared with AdoA1Rf/f mice (n = 5; waking mean, 50 ± 8.4%; SWS mean, 41.4 ± 6.4%; REM mean, 8.6 ± 2.4%). As previously reported for AdoA1R constitutive gene deletion mutants (Stenberg et al., 2003), we did not detect a significant phenotype with respect to the percentage of total time spent in any one of the three sleep/waking states.

The loss of AdoA1R expression resulted in a decrease of the average SWA power (frequency range of 3.0–4.5 Hz) over the baseline recording period (AdoA1Rf/f SWA, 59.3 ± 2.2 μV²/epoch; AdoA1Rf/f SWA, 49.8 ± 1.1 μV²/epoch; p < 0.001) (Fig. 3B). This genotype difference in SWA was amplified during SWS, the state in which SWA predominates (AdoA1Rf/f SWS-SWA, 83.6 ± 2.8 μV²/epoch; AdoA1Rf/f SWS-SWA, 69.5 ± 2.3 μV²/epoch; p < 0.0005) (Fig. 3C) and was absent during waking (AdoA1Rf/f waking–SWA, 40.8 ± 12.5 μV²/epoch; AdoA1Rf/f waking–SWA, 37.2 ± 8.3 μV²/epoch) (Fig. 3B). The ability to increase SWA from waking to SWS was significantly attenuated by the loss of AdoA1R expression (AdoA1Rf/f, increased SWA by 110 ± 6.22%; AdoA1Rf/f, 82.1 ± 7.96%; p = 0.01) (Fig. 3C). Thus, even under baseline sleep conditions, SWA expression was attenuated during SWS in animals with disrupted AdoA1R expression.

An altered SWA power frequency band distribution was observed in mice with a mutated retinoic acid receptor β gene during SWS primarily in the 3.0–4.5 Hz range (Maret et al., 2005). The frequency-specific contribution to the power of EEG signal during SWS was assessed for AdoA1Rf/f mutants and AdoA1Rf/f mice. No change in the power distribution in the SWA frequency range of 0.5–15.0 Hz between the two groups was apparent (Fig. 4).

Restricted sleep response: acute 4 h sleep deprivation

The reduced SWA in the AdoA1Rf/f mice may have resulted from aberrant homeostatic control in relation to waking duration. However, the AdoA1Rf/f mice had spontaneous waking periods of similar length as the waking periods of AdoA1Rf/f mice (average duration of waking for AdoA1Rf/f, 2.3 ± 0.4 min; AdoA1Rf/f, 2.1 ± 0.1 min). They nevertheless expressed significantly less SWA during the ensuing SWS periods (Fig. 3C). The magnitude of the SWA power is closely correlated with waking duration (Dijk et al., 1990; Franken et al., 2001). If these correlations are causally related, then our observations of the genotype-dependent difference in SWA are consistent with an altered, less effective SWA-inducing feedback.

To test for a genotype-dependent change in the relationship between a controlled waking duration (i.e., forced waking) and SWA, both genotypes of mice (n = 7 per genotype) were maintained awake on a slowly moving treadmill for a 4 h period, followed by a 2 h undisturbed period (treadmill was turned off).
Figure 4. SWS EEG power distribution by genotype. Power distribution (calculated as a percentage of total EEG power for each 1.0 Hz bin) in the frequency range from 0.5 to 15 Hz during SWS did not significantly differ between AdoA1R+/+ and AdoA1R−/− mice (n = 5 per genotype). Thus, although AdoA1R−/− animals show decreased SWA in the 3.0 – 4.5 Hz band, this frequency range (3.0 – 4.0, 4.0 – 5.0 Hz) makes up the same relative amount of the total power during SWS. Error bars indicate SEM.

Figure 5A shows an example of raw EEG signals from both groups during baseline and after enforced waking conditions. Both genotypes responded to the enforced waking with increased SWA during the 2 h undisturbed period, but the magnitude of the SWA during SWS was significantly greater for the AdoA1R+/+ genotype compared with the AdoA1R−/− group (AdoA1R+/+, 69 ± 0.6 μV²/epoch; AdoA1R−/−, 59.3 ± 3.0 μV²/epoch; p = 0.01) (Fig. 5B). However, there was no difference in percentage time in SWS between genotypes (AdoA1R+/+, 45.5 ± 4%; AdoA1R−/−, 50.2 ± 5.8%). This indicates an adenosine-mediated role in the rebound SWA response to prolonged waking acts via the AdoA1R.

The restricted sleep response: chronic sleep restriction, sleep duration, and slow-wave activity

In mice, 4 h of sleep deprivation did not result in any significant change in SWS time, but SWA during SWS was greatly enhanced. To test whether chronic sleep restriction in mice can be functionally compensated by a rebound SWA increase in an AdoA1R-dependent manner, SWS time and SWA were assessed in both genotypes under conditions of chronic sleep restriction.

The opportunity to sleep was restricted to a 2 h period per 6 h cycle by enforcing waking for 4 h with a slowly moving treadmill. The 6 h cycle (4 h TM on and 2 h TM off) was repeated for 48 h (eight complete cycles). Notably, there was no change in SWS time during spontaneous sleep periods (baseline) compared with the same time during TM off for either AdoA1R+/+ mice (n = 5; 44.9 ± 2.3% baseline; 49.2 ± 3.2% restricted sleep TM off) or for AdoA1R−/− mice (n = 5; 44.6 ± 2.2% baseline; 49.9 ± 4.6% restricted sleep TM off), nor was there any difference in SWA time between genotypes. Mice, of either genotype, spent 10.1 h per d in SWS under baseline conditions, whereas under the sleep restriction paradigm only 3.5 h per day was spent in SWS, a 65% reduction in SWS time.

In contrast to SWS time, SWA recorded during the 2 h TM-off phase, compared with the baseline sleep day (recorded at the same circadian time), showed an increase over baseline magnitude for both genotypes. SWA was significantly lower for the AdoA1R−/− mice on every cycle during the TM-off phases (AdoA1R−/− average, 51.3 ± 2.1 μV²/epoch; average AdoA1R+/+, 70.8 ± 2.4 μV²/epoch; p < 0.001) (Fig. 6A, C). Thus, during chronic restricted sleep, SWA, regardless of state, was attenuated in an AdoA1R-dependent manner with no change in SWS time.

Because SWA predominates during SWS, SWA power recorded during SWS during sleep restriction was compared across genotype. The largest SWA difference between genotypes was observed under these conditions (AdoA1R+/+, 106.2 ± 3.4 μV²/epoch; AdoA1R−/−, 65.9 ± 3.2 μV²/epoch), showing ~30 μV²/epoch difference (p < 0.0001) (Fig. 6B, C). The percentage change in SWA from the TM-on phase when the animals were awake to SWS during the TM-off phase (calculated as [SWS_SWA TM off−SWS TM on]/SWS TM on) was, for AdoA1R+/+, 254 ± 11%; for AdoA1R−/−, 139 ± 12.3%; significant at p < 0.0002 (Fig. 7). This suggests the AdoA1R is needed for the full expression of the rebound SWA response during sleep restriction.

These observations suggest that the AdoA1R may be particularly important to the increase in SWS from waking to SWS when sleep time is restricted, compared with our baseline unrestricted conditions. Indeed, AdoA1R+/+ animals showed a significant increase in SWS SWA after forced waking compared with baseline SWS SWA (baseline, 83.6 ± 2.8 μV²/epoch; TM off, 106.2 ± 4.8 μV²/epoch; p < 0.001) (Fig. 6A, C). Thus, during chronic restricted sleep, SWA, regardless of state, was attenuated in an AdoA1R-dependent manner with no change in SWS time.
μV^2/epoch; \( p < 0.001 \) (Fig. 7), whereas there was no significant increase in SWS SWA in AdoA1Rf/f animals (baseline, 69.5 ± 2.3 μV^2/epoch; TM off, 65.9 ± 3.9 μV^2/epoch).

We compared the increase in SWA activity from waking to SWS under baseline conditions (Fig. 3C) to the increase in SWA from waking to SWS under sleep-restricted conditions (Fig. 7, left pair of bars). Notably, AdoA1Rf/f mice were able to further increase SWA from waking to SWS in sleep-restricted conditions compared with baseline conditions. This additional increase was significantly greater than that observed with mice with disrupted AdoA1R activity (AdoA1Rf/f, 42.3 ± 12.8%; AdoA1R, R⁻/⁻, 9.81 ± 7.37%; \( p = 0.005 \) ) (Fig. 7, right pair of bars). This change was calculated as follows: \([\text{increase of SWA}_{\text{restricted}} - \text{increase SWA}_{\text{baseline}}]/\text{increase of SWA}_{\text{baseline}}\). The findings are consistent with a ceiling effect on SWA during SWS in AdoA1R R⁻/⁻ mice, suggesting that additional enhancement of SWA in sleep-restricted conditions requires the AdoA1R.

Finally, we compared the percentage change in SWA during SWS from baseline to sleep restriction under acute (one 6 h cycle) (Fig. 5) and chronic (eight 6 h cycles) (Fig. 6) sleep restriction. There was no difference in percentage change between acute and chronic sleep restriction in either group (data not shown).

These data suggest the AdoA1R is necessary for the compensatory SWS-specific rebound that follows sleep restriction. This may indicate the possibility of functional implications for the compensatory SWA increase.

**Working memory performance and sleep restriction**

A recent study suggests that an increase in SWA during SWS follows new learning, and improved performance is correlated with this increase in SWA (Huber et al., 2004). Because the AdoA1R R⁻/⁻ mice show a selective deficit in the SWA response to restricted sleep, we examined their performance on a working memory task in comparison with AdoA1R R⁻/⁻ mice, before, during, and after 48 h of sleep restriction. Ten animals per genotype were examined on a working memory task. These animals were not connected to the EEG/EMG recording system.

There was no difference in working memory performance between genotypes before testing (AdoA1R R⁻/⁻, 1.6 ± 0.3 revisit errors/trial; AdoA1R, R⁻/⁻, 1.6 ± 0.3 revisit errors/trial) (Fig. 8A). The two genotypes were then sleep restricted on the treadmill with the 4 h TM-on/2 h TM-off protocol for three full cycles (18 h). At the beginning of the fourth cycle, they were tested on the eight-arm maze working memory task (during the time when waking was restricted). They were then returned to the sleep restriction protocol in time to begin the 2 h TM-off phase of the fourth cycle (hours 18–24). Twenty-four hours later, at the beginning of the eighth cycle, the mice were tested again for the working memory task, and then allowed to sleep ad libitum for another 24 h when they were tested again for recovery (Fig. 8A). During the sleep restriction, the AdoA1R R⁻/⁻ mice showed a significant increase in the number of revisit errors (4.1 ± 1.3 errors;
SWA response. The attenuation of SWA is associated with compromised working memory-dependent performance, indicative of a functional role for AdoA₁R-dependent SWA homeostasis in maintaining this cognitive performance when sleep is restricted.

Several studies indicate that the expression of SWA is under genetic control (Franken et al., 1998, 2001; Wisor et al., 2002; Maret et al., 2005), but the specific molecular, neurochemical, and physiological mechanisms responsible for this control remain mostly uncharacterized. At the cellular level, AdoA₁Rs mediate an increase in GIRK channel currents and a decrease in hyperpolarization activated currents, both of which facilitate the oscillations that underlie SWA (Pape, 1992). In addition, activation of AdoA₁Rs on presynaptic terminals reduces synaptic glutamate release, thereby reducing excitatory drive (Brambilla et al., 2005) and allowing electrophysiological relaxation of neurons toward a more hyperpolarized membrane potential needed for the emergence of endogenously generated SWA oscillations (McMorrow and Pape, 1990). Application of exogenous adenosine and AdoA₁R agonists either systemically or locally to the diencephalon increases SWS (Radulovacki, 1985) and SWA recorded from the surface EEG (Benington and Heller, 1995; Benington et al., 1995).

An indirect AdoA₁R-mediated mechanism to increase SWS and SWA in response to waking may result from a local increase in extracellular adenosine in cholinergic arousal centers. Increased glutamate-mediated excitatory input to neurons of these centers, associated with prolonged waking, increases AdoA₁R activation (Rainnie et al., 1994; Arrigoni et al., 2001; Brambilla et al., 2005). This activation reduces arousal center activity, acting at both presynaptic and postsynaptic inhibitory AdoA₁Rs (Rainnie et al., 1994; Arrigoni et al., 2001), and accordingly decreases cholinergic tone in the target sites of these centers, including the thalamocortical system. The decrease in cholinergic tone facilitates SWS as observed when adenosine reuptake is blocked (increasing endogenous adenosine concentration) in the cholinergic basal forebrain (Porkka-Heiskanen et al., 1997). Thus, AdoA₁R-mediated actions can potentially facilitate the increase in SWA by two complementary mechanisms during prolonged waking (Rainnie et al., 1994; Porkka-Heiskanen et al., 1997; Portas et al., 1997; Arrigoni et al., 2001). However, the requirement for AdoA₁R-mediated increase in homeostatic SWA has not previously been demonstrated.

Adenosine is the endogenous ligand for the AdoA₁R and its extracellular concentration increases whenever the ratio of metabolite availability to metabolite demand decreases (McClinn and Poll, 1986; Greene and Haas, 1991; Dunwiddie and Masino, 2001). An AdoA₁R-mediated inhibitory tone, present under physiological conditions, is positively modulated with a slow time constant in response to maintained increases in excitatory synaptic glutamate release as occurs with maintained waking (Brambilla et al., 2005). Because extracellular adenosine concentration reflects the intracellular concentration that is maintained in equilibrium with ATP/ADP ratio by adenosine kinase (Munchmore et al., 2006) in both glia and neurons (Studer et al., 2006), the AdoA₁R inhibitory tone reflects the intracellular metabolic state of nervous tissue. Accordingly, intracellular metabolic state can modulate the functionally relevant enhancement of synchronized SWA during the SWS state. Although the loss of AdoA₁Rs in the conditional gene deletion used in this study are exclusively neuronal (Tsien et al., 1996), the source of the adenosine may include both neurons and glia (Pascual et al., 2005; Studer et al., 2006) and reflect the metabolic state both kinds of neural tissue.

The data presented here suggest that, under physiological con-
ditions during sleep restriction, AdoA1R activation is needed for the full expression of a homeostatic increase of SWA. Nevertheless, SWA still predominates during SWS in the absence of AdoA1R activation because it was clearly observed in AdoA1R^{-/-} mice. Furthermore, in a mutant mouse with constitutive deletion of the AdoA1R gene, SWA was not altered in spontaneously sleeping mice and mice sleep deprived by gentle handling (Stenberg et al., 2003). These findings are most consistent with a modulatory role for AdoA1R activation in SWA expression.

Additionally, the working memory deficits seen in the AdoA1R^{-/-} mice under sleep restriction may be attributable to factors other than the decreased SWA, such as stress or changes in other frequency bands. It is unlikely that a stress effect would only appear in the knock-out animals because all mice had the same amount of forced waking at the same belt speed. We cannot rule out differences in other frequency bands in the AdoA1R^{-/-} animals; however, the greatest changes in frequency during SWS after sleep restriction are in the SWA frequency band (Borbély, 1982). Thus, it is reasonable to conclude that the working memory effects seen by knocking out the AdoA1 receptor are most likely attributable to the concurrent changes in SWA.

The effect of the inducible deletion of the AdoA1R gene in CNS was remarkably selective for an attenuated rebound response in SWA to sleep restriction. SWA was only slightly attenuated in baseline conditions, and the percentage of time spent in SWS in either baseline or during sleep restriction was not affected by the gene deletion. This selectivity offered an opportunity to dissociate changes in SWA from changes in SWS time because AdoA1R^{-/-} mice had an attenuated homeostatic SWA response but an unchanged SWS time in our sleep restriction paradigm. The ability to further increase SWA expressed during SWS under baseline conditions to the larger amplitude of SWA expressed during SWS under the sleep restriction conditions was marked in the AdoA1R^{0/0} mice and almost completely absent in the AdoA1R^{-/-} mice (a >40% compared with <10% additional increase). This suggests an AdoA1R-dependent compensatory increase in SWA.

AdoA1R^{-/-} mice could perform a working memory cognitive task normally under baseline conditions, but performance was compromised under conditions of sleep restriction. This deficit in performance was associated with the deficit in the compensatory SWA response, and the deficit was recovered when sleep was no longer restricted. Thus, loss of AdoA1R function compromises working memory performance but only under conditions involving the reduction of rebound SWA, consistent with a role for rebound SWA in cognitive performance.

Notably, attenuated SWA did not affect a hippocampal-dependent, paired associates learning task used to assess episodic memory. Mice learned that one of two odor cues was rewarded in one context, and that in another context the reward assignment was reversed. After training, acquisition of a new odor/context pair was then tested during 5 d of the 4 h TM-on, 2 h TM-off sleep restriction condition. There was no difference in performance (percentage of correct trials) between groups (B, C). There was no difference in the weight (B) or the time to task completion (C) between groups, suggesting that there was no difference in motivation or in sensory/motor ability to perform the task. Error bars indicate SEM.

Figure 8. SWA-dependent deficit in working memory performance. Sleep restriction reduces working memory capacity in animals with conditional loss of AdoA1R gene expression, but it has no effect on those with intact AdoA1R expression. A, Pooled data for each genotype during baseline and sleep restriction (TM) conditions showed a performance deficit (increase in revisit errors) for AdoA1R^{-/-} animals, but no deficit for AdoA1R^{0/0} animals. The deficit was reversed after a 3 d return to baseline sleeping conditions (recovery). B, C, There was no difference in the weight (B) or the time to task completion (C) between groups, suggesting that there was no difference in motivation or in sensory/motor ability to perform the task. Error bars indicate SEM.

Figure 9. Sleep restriction did not affect acquisition of a hippocampal-dependent appetitive, paired associates learning task used to assess episodic memory. Mice learned that one of two odor cues was rewarded in one context, and that in another context the reward assignment was reversed. After training, acquisition of a new odor/context pair was then tested during 5 d of the 4 h TM-on, 2 h TM-off sleep restriction condition. There was no difference in performance (percentage of correct trials) between groups (white/black bars); however, both genotypes showed significant improvement across days (gray cross-hatched bars). Error bars indicate SEM.
may be less critical for the performance of this task for the same reason (i.e., all cues are always present, a condition that contrasts with the eight-arm working memory task). Our observations suggest that the neural processes involved in hippocampal-dependent learning and episodic memory (Rajji et al., 2006) are less sensitive or even insensitive to the AdoA1R loss of function and the attenuated SWA compared with those required for normal working memory capacity. It is conceivable that any negative effect of the disrupted rebound SWA response was opposed by the loss of AdoA1R inhibitory tone in the hippocampus, although these same speculated opposing effects ought to apply to the neural mechanisms involved in performance of the working memory task as well, and this was not the case. Synaptic plasticity responsible for the acquisition, retention, and consolidation involved in the paired associate task was less sensitive than the neural mechanism(s) needed to perform the eight-arm maze working memory task.

With respect to the mechanisms responsible for performance of the working memory task, the observed reversibility of the sleep restriction-induced deficit in AdoA1R−/− mice suggests that synaptic plasticity and/or consolidation processes were not involved. A similar kind of selective loss of function of working memory compared with reference memory has been described as resulting from the deletion of the GluR1 subunit of the AMPA receptor (Schmitt et al., 2003) consistent with a greater sensitivity of working memory to perturbations of glutamatergic function rather than mechanisms directly related to synaptic plasticity. A major difference in neuronal function with respect to working memory performance compared with episodic memory is that the former requires sustained, selective, circuit activity of the prefrontal cortical circuits (Constantinidis et al., 2002; Lee and Kesner, 2003; Wang et al., 2004). The loss of AdoA1R function and the associated rebound SWA may be one of the factors that selectively compromise the more metabolically demanding activities such as those involved in sustained circuit activity under conditions that produce rebound SWA-like restricted sleep and thus affect working memory performance.

The nature of sleep function remains enigmatic (Greene and Siegel, 2004), especially because the molecular mechanisms controlling a homeostatic increase in SWA are not well understood. It has been suggested that sleep function is involved in the consolidation of learning (Walker and Stickgold, 2004; Stickgold and Walker, 2005) or in the global homeostasis of synaptic plasticity of learning and memory (Vyazovskiy et al., 2008), based on observations of performance with or without intervening episodes of sleep. Nevertheless, whether these sleep dependent changes are attributable to a direct modulation of synaptic plasticity or to an indirect outcome from some other process that impinges on synaptic plasticity remains to be established. Indeed, there is considerable evidence consistent with a role in metabolism, feeding, and sleep based on known modulators of sleep that involve metabolism and feeding, including NPAS2 (Rutter et al., 2001; Dudley et al., 2003), clock (Naylor et al., 2000; Turek et al., 2005), leptin (Laposky et al., 2006), orexin/hypocretin (Chemelli et al., 1999; Lin et al., 1999; Willis et al., 2001) and adenosine (Brambilla et al., 2005).

Together, our findings suggest that metabolically associated feedback signals of waking, involving AdoA1R, activation, influence homeostatic sleep functions in the CNS. The homeostatic sleep function requires rebound synchronized SWA, as suggested by the observation that loss of AdoA1R function and the selective attenuation of this homeostatic sleep response reduces working memory capacity.

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