Learning-Dependent, Transient Increase of Activity in Noradrenergic Neurons of Locus Coeruleus during Slow Wave Sleep in the Rat: Brain Stem–Cortex Interplay for Memory Consolidation?

Memory consolidation during sleep is regaining attention due to a wave of recent reports of memory improvements after sleep or deficits after sleep disturbance. Neuromodulators have been proposed as possible players in this putative off-line memory processing, without much experimental evidence. We recorded neuronal activity in the rat noradrenergic nucleus locus coeruleus (LC) using chronically implanted movable microelectrodes while monitoring the behavioral state via electrocorticogram and online video recording. Extracellular recordings of physiologically identified noradrenergic neurons of LC were made in freely behaving rats for 3 h before and after olfactory discrimination learning. On subsequent days, if LC recording remained stable, additional learning sessions were made within the olfactory discrimination protocol, including extinction, reversals, learning new odors. Contrary to the long-standing dogma about the quiescence of noradrenergic neurons of LC, we found a transient increase in LC activity in trained rats during slow wave sleep (SWS) 2 h after learning. The discovery of learning-dependent engagement of LC neurons during SWS encourages exploration of brain stem–cortical interaction during this delayed phase of memory consolidation and should bring new insights into mechanisms underlying memory formation.

Keywords: cortical replay, electrophysiology, memory consolidation, neuromodulation, noradrenaline, slow wave sleep

Introduction

The notion that memories are reinforced during sleep gains increasing acceptance due to a new wave of studies showing improvements in performance after sleep or deficits after sleep disturbance (Gais and Born 2004a; Huber et al. 2004; Stickgold 2005). A hallmark feature of the sleep/wake cycle is systematic variation in activity of neuromodulatory neurons. Little is known about the functional significance of this variation, although neuromodulators are beginning to receive consideration as possible players in this putative off-line memory processing (Payne and Nadel 2004; Gais and Born 2004b; Colgin and Moser 2006; Walker and Stickgold 2006). For example, there is evidence that low levels of acetylcholine associated with slow wave sleep (SWS) are beneficial for off-line memory processing (Gais and Born 2004b).

Many years ago, Kety proposed that noradrenaline (NE) might promote memory by selectively reinforcing neural networks activated during an aroused state, suggesting that “release of catecholamines may favor consolidation of learning by stimulating protein synthesis...” through action of cyclic adenosine monophosphate on protein kinases (Kety 1972, p. 383). Subsequent research has, indeed, shown a consistent time window 3–6 h after learning when memory processes are vulnerable to interference with this intracellular signaling pathway (Bernabeu et al. 1997). The cAMP cascade is activated by a family of metabotropic receptors including the beta-noradrenergic receptors. Injection of beta-receptor antagonists into the lateral ventricles (Roulet and Sara 1998) or prelimbic cortex (Tronel et al. 2004) 2 h after learning, induced robust amnesia in rats, whereas injections immediately after training had no effect, suggesting engagement of the noradrenergic system in a late stage of memory consolidation. The question remains as to whether activation of locus coeruleus (LC) actually occurs at this time window. At first glance this would seem unlikely, because rats are notoriously nocturnal, spending most of their postlearning time sleeping, when activity in LC neurons is markedly diminished (Roussel et al. 1967; Hobson et al. 1975; Aston-Jones and Bloom 1981). Nevertheless, we were encouraged to consider the role of LC in a late phase of memory consolidation because, in addition to the delayed action of beta-noradrenergic antagonists, microdialysis revealed a transient increase in NE release in prelimbic cortex, 2 h after learning (Tronel et al. 2004).

If this transient noradrenergic activation occurs during SWS it would be of particular functional significance because reactivation of neuronal ensembles, previously active during wakefulness, occurs during this state (Pavlides and Winson 1989; Wilson and McNaughton 1994; Skaggs and McNaughton 1996; Pennartz et al. 2004). Neuromodulators have not, as yet, been implicated in this putative off-line memory processing, but there has been recent speculation concerning their possible role (Colgin and Moser 2006). The unveiling of a learning-dependent engagement of LC neurons during SWS would encourage exploration of brain stem–cortical interaction during this delayed phase of memory consolidation to bring new insights into mechanisms of cortical plasticity underlying memory.

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River Laboratory, Le Genest-St-Isle, France), weighing 350–400 g were used. All procedures followed the 1986 European Communities Council Directive and Ministère de l’Agriculture et de la Foret, Commission Nationale de l’Experimentation Animal decree 87848.

Surgery

The rat was anesthetized with sodium pentobarbital (50 mg/kg ip, supplemented as necessary) and mounted in a stereotaxic instrument with the incisor bar lowered to an angle of ~14°). Two tungsten...
microelectrodes (−1 MΩ; FHC, Bowdoinham, ME), glued together and mounted on a movable drive were implanted into LC (lambda: AP = −4.0, L = 1.15) at a depth of about 5.3 mm under electrophysiological control. LC cells were identified as lying just under the fourth ventricle, firing at about 1 Hz, with broad action potentials. A paw-pinch elicited a phasic response from the entire population of LC cells, followed by prolonged inhibition. The general surgical procedures and further details of movable microelectrode implantation into LC have been described elsewhere (Sara and Segal 1991; Herve-Minvielle and Sara 1995).

Two stainless steel screw electrodes were placed over the left prefrontal cortex (Bregma: AP = ±3.5, L = 0.5; reference: AP = +0.5, L = 0.5) for electroencephalography (EEG) recording. In 2 rats electromyogram (EMG) activity was recorded from the dorsal neck muscles with insulated multistranded wires to validate the sleep-scoring procedure based on EEG and video recordings. When EMG was not available, the rat behavioral states, particularly REM episodes, were confirmed by video (Supplementary Fig. 1). All electrode wires were secured into 6-pin connector (Plastics One Inc., Roanoke, VA) fixed on the rat skull.

**Electrophysiological Recordings**

Rats were connected to the recording system via a rotating cable allowing free movement within the recording chamber (25 × 25 × 50 cm in size). EEG, EMG, and LC unit activity were recorded continuously for 3 h, amplified and filtered using a differential amplifier (A-M Systems, 5000, WA) and digitized using Power1401 converter and Spike2 data acquisition software (Cambridge Electronic Design, Cambridge, UK). EEG signals were amplified (× times one thousand), filtered between 0.1 and 300 Hz and sampled at 1 kHz. EMG was amplified (× times one thousand), filtered at 30–300 Hz and sampled at 1 kHz. LC activity was recorded through custom-build preamplifiers (gain × 1), then amplified (× 10k) and filtered at 300 Hz–3 kHz and sampled at 30 kHz.

Unit activity was monitored online and spikes with signal-to-noise ratio greater than 2:1, were used to create templates online using Spike2 software. Criteria used for identifying LC neurons were the following: 1—broad spike widths (~600 μs); 2—regular low firing rate (1–2/s) during the quiet awake state; 3—response to acoustic stimuli with a brief short-latency excitation followed by prolonged inhibition; 4—absence of firing during REM episodes (Hobson et al. 1975; Foote et al. 1980; Aston-Jones and Bloom 1981; Sara and Segal 1991). If the unit discharge did not meet criteria for an LC neuron, the electrode was lowered at 40-μm increments to reach a reliable LC signal.

A video camera (Quickcam, Logitech, Moulin du Choc, Switzerland) was mounted on the top of the recording chamber, the image synchronized with electrophysiological recording. Data were stored on a PC for off-line analysis. Recordings were made between 10 A.M. and 3 P.M. during the light period, when rats spend most of their time sleeping. All recordings and behavioral manipulations for each individual rat were made at approximately the same time of day to avoid the effects of circadian rhythm fluctuations in activity of noradrenergic neurons and EEG.

**Behavioral Procedures**

After a 1-week recovery from surgery, rats were kept on a food-restricted diet (20 g per day; body weight not less than 80% of free-food weight) and habituated to the recording chamber and plugging procedure. The quality of unit recording was checked daily. If the signal met criteria for an LC cell (described above) and if units were at least 2× background activity, the rat was submitted to a behavioral session followed by a 3 h recording of LC unit activity, EEG, EMG, with added video monitoring of behavior (Supplementary Fig. 1) in the recording chamber. If LC neurons could not be identified, the electrodes were slowly advanced in 40-μm increments until a stable recording meeting the criteria for an LC unit was obtained.

A baseline recording was performed in naive rats in a familiar environment (recording chamber). On the following days, rats were subjected to habituation and pretraining procedures. The habituation procedure consisted in rat exploration of the experimental box with randomly distributed reward during a 10-min session. As the pretraining procedure, rats were progressively shaped to dig for reward in an odor-free gravel-filled cup in a neutral (nonlearning) environment. Then rats were trained to forage for reward by nose poking in sponges or digging in a gravel-filled cup. A specific target odor was associated with a palatable reward (chocolate or honey flavored puffed rice cereal to which the rat had been previously accustomed). The sponge task is described in detail elsewhere (Tromel and Sara 2002). Three sponges, each with a hole in the middle and impregnated with a specific odor, were placed in the corners of the experimental box. Reward was placed at the bottom of the hole of the sponge with the designated target odor. A nose poke into the hole obtained reward. Placement of the target odor varied randomly in each trial. The digging task was a slightly modified version of the sponge task and is described in detail elsewhere (Eschenko et al. 2006). Four cups filled with gravel, each containing a powdered spice with a distinct odor were placed in the corners of the experimental box. Both learning procedures (sponge and digging) were performed in the same experimental box (1 m × 1 m × 0.6 m). Rats were allowed 5–10 massed trials with 1- to 2-min intertrial interval to make an odor-reward association (total training duration around 30 min).

After the baseline and postlearning recordings were completed, rats were submitted to additional learning situations on subsequent days if reliable LC recording could still be obtained (Table 1). These included retrieval, extinction, and new learning. Retrieval tested memory retention in the situation identical to learning; new learning required a new odor-reward association; extinction required learning that reward was no longer associated with the target odor.

**Data Processing and Statistical Analysis**

Sleep/awake states were characterized based on EEG, EMG, and video recording; 10-s epochs were used for scoring by an experienced...
observer (O.E.) according to standard criteria (Bjorvatn et al. 1998). Awake state was marked by the presence of low-amplitude fast activity associated with increased EMG tonus. SWS was identified by continuous high-amplitude delta activity at around 200 μV (or at least 2 times higher in amplitude than EEG during the awake state), the power spectrum showing a predominance of delta and absence of theta frequencies and regular appearance of spindles. The minimum duration of an individual SWS epoch was restricted to 30 s of continuous and regular delta activity with at least one spindle per 10 s and not interrupted by micro-awakenings. This scoring procedure enabled us to take SWS episodes maximally resembling human non-REM sleep stage 2 (De Gennaro and Ferrara 2003). REM sleep was characterized by dominant theta activity, low-voltage fast activity and absence of EMG tonus; sleep posture was additionally verified by video (Supplementary Fig. 1).

To quantify the average amplitude of the delta waves, EEG was first low-pass filtered with a Finite Impulse Response (FIR) filter of 30 Hz and the resulting signal was down sampled to 200 Hz. Subsequently this signal was low-pass filtered with an FIR filter of 3.5 Hz. After filtering the root mean square (RMS) was calculated at every time point using a time window of 1 s and finally the RMS signal was smoothed with a moving average of 10 s. The average amplitude of the resulting signal (DeltaRMS) was calculated for each SWS episode.

To identify the spindle activity a FIR band-pass filter of 12–15 Hz was used; an automatic spindle detection algorithm was used (Eschenko et al. 2006).

The LC recording was processed off-line. Various routines based on the principal component analysis and specific wave form measurements were applied to isolate single units. Wave forms of units were displayed as overlays as shown at the bottom of Figure 2A and interspike interval histograms were generated. To be classified as a single unit, no more than 1% of interspike intervals could be less than 2 ms. Most importantly, all action potentials had the electrophysiological response characteristics of LC neurons, as described above, and all cells were silent during REM sleep. Finally, only recordings from rats having clear electrode placement in LC as determined by both Cresyl Violet and tyrosine hydroxylase staining were included in the analysis (Fig. 1). Using these strict criteria, we recorded LC units from 8 rats during total of 34 recording sessions (Table 1).

**Statistical Analyses**

To statistically evaluate the temporal dynamics of LC activity, mean LC firing rate during each SWS episode (minimum 30 s of uninterrupted sleep) was normalized to the mean rate during awake state of the same unit in the same recording session and averaged over 30-min time intervals. Data were subjected to analysis of variance (ANOVA) for repeated measures with 6 time series as a repeated measure and using unit in the same recording session and averaged over 30-min time intervals. A subsequent analysis included all postlearning recording sessions (retrieval, extinction, new learning; n = 17; see Table 1). Post hoc comparisons were made using the Newman-Keuls test. The threshold of 2 SD above the mean of the LC firing rate during the SWS episodes of the first hour of recording was used to identify time windows with elevated LC activity. This statistical criterion denotes a probability at the 0.05 level that the change is significant. EEG power spectra were compared using a paired t-test with Bonferroni correction.

### Results

Eight rats with histologically confirmed electrode placements in the LC nucleus (Fig. 1) contributed to total of 34 recording sessions (Table 1). One rat with a confirmed LC electrode placement (Rat 2 in Table 1) did not have reliable LC recording in any of the learning sessions and thus contributed to the baseline and habituation analyses only.

As reported in previous experiments (Tronel et al. 2002; Tronel et al. 2004) rats, when they learn, learn this task rapidly within a single training session, reaching an asymptotic performance in terms of errors and latencies. Therefore, although there is an obvious advantage of this behavioral paradigm in terms of fast and reliable odor-reward association learning, it is not possible to correlate behavioral performance or rate of learning of individual rats with subsequent electrophysiological activity. A certain number of rats never learned the discrimination and solved the problem with random foraging (e.g. rat 1 in the present series; see Table 1).

None of the training procedures resulted in any global change in sleep/awake pattern during the first 3 h after behavioral manipulation. Number of AWAKE, SWS, and REM episodes, total time or average duration of each episodes were not significantly different from the baseline (paired t-tests; Table 2).

The mean firing rates of all identified LC neurons (n = 34) obtained from 8 rats during AWAKE, SWS, and REM episodes showed the variation over the sleep/awake cycle, which is characteristic for noradrenergic neurons (quiet AWAKE: 1.15 ± 0.12 spikes/s; SWS: 0.47 ± 0.11 spikes/s; REM: 0.11 ± 0.04 spikes/s; mean ± SEM).

The striking result was the consistent transient appearance of an increase of LC activity during postlearning SWS around
2 h after the end of the learning session. Figure 2A illustrates this phenomenon with extracts from an individual recording session, with the top line showing individual LC action potentials during 2 min of recording during quiet AWAKE (left panel), SWS shortly after learning (middle panel), and SWS 114 min after learning (right panel). The raw EEG and its corresponding power spectrum for the same time intervals are shown below the LC activity on Figure 2A, illustrating that,

Figure 2. (A) Simultaneous recording of LC unit activity and EEG after a learning session. Left panel: 2-min recording during a quiet awake episode, 14–16 min after the end of the learning session. The quiet awake state is depicted by the low amplitude, fast activity in the EEG and the theta peak in the power spectrum (bottom left of the panel). LC firing rate is typical of this behavioral state (1.42/s); individual LC spikes are shown at the top, with the same spikes, at a slower time scale to depict the waveform, overdrawn at the bottom right. Middle panel: 2-min recording during SWS episode, 90–92 min after learning. The EEG is characterized by high-amplitude delta waves, the power spectrum at the bottom left showing a predominance of delta and absence of theta frequencies. Firing rate of the LC cell is diminished (0.23/s) as seen in the individual spikes at the top and the overdrawn waveform forms of the same cell, depicted at a slower time scale. Right panel: 2-min recording during SWS episode, 114–116 min after learning. The EEG and its power spectrum is the same as during the earlier SWS episode shown on the middle panel. Firing rate of the LC cell (1.67/s) is comparable to the quiet awake state, as seen in the individual spikes at the top and the overdrawn waveforms at a slower time scale, bottom right. (B) Schematic representation of the onset and duration of elevated LC activity during successive SWS epochs, plotted for each of the 13 postlearning recordings showing a significant increase of LC firing rate; black bars: learning, hatched bars: extinction; striped bars: retrieval. (C) Mean LC firing rate during 20 sequential SWS episodes, centered around the first SWS episode (arrow) showing a significant increase of SWS-associated LC activity (mean ± SEM). The data are taken from the 13 postlearning recordings depicted at the Figure 2B. Note an abrupt onset from one episode to another and the gradual decrease over subsequent SWS episodes.

| Table 2 |
|---|---|---|
| Sleep architecture for 3 h after baseline and after learning |
| Number of episodes | Total time (s) | Episode duration (s) |
| | AWAKE | SWS | REM | AWAKE | SWS | REM | AWAKE | SWS | REM |
| Baseline, n = 7 | 14.0 ± 1.8 | 43.3 ± 5.3 | 6.1 ± 1.1 | 3239.6 ± 407.2 | 4658.5 ± 440.0 | 833.7 ± 126.6 | 256.3 ± 47.4 | 117.5 ± 17.5 | 147.8 ± 14.2 |
| Learning, n = 17 | 13.2 ± 0.8 | 37.7 ± 2.4 | 5.6 ± 0.9 | 3704.4 ± 327.6 | 4229.0 ± 201.0 | 793.6 ± 124.1 | 297.6 ± 31.9 | 118.2 ± 7.8 | 143.4 ± 8.3 |

Note: There were no significant differences in the number of episodes, the total time spent in different states or in the duration of an episode.
despite augmentation of LC activity, the cortical EEG reflected the typical SWS state with a predominant high-amplitude delta activity. Additional examples of the data illustrating the same phenomenon obtained from different rats and after different learning situations are shown on Supplementary Figure 2A-E.

The delayed elevated LC firing during SWS was significant in 13/17 postlearning sessions (Table 1; sessions highlighted in bold font). The onset (99.4 ± 7.1 min) and total duration (22.9 ± 4.3 min) of the LC activation varied across recordings as shown on the Figure 2B. The combined data for the 13 sessions in which there was a significant postlearning LC activation is shown on the Figure 2C. Each bar shows the average LC firing rate during the sequential SWS episodes before and after the first SWS episode where a significant LC activation was detected (arrow). It is important to underline that such elevations during SWS were never seen in any of the baseline recording sessions. It was also not present in other control conditions with no associative learning, that is, after habituation to the experimental box with randomly available reward or after procedural training for digging (Table 1; Fig. 3).

The temporal dynamics of SWS-associated LC activity was first analyzed for only the recordings after the first formal learning session from each rat in order to allow the data to be submitted a 2-way randomized block ANOVA for repeated measures, in a strict within-subject design (Table 1; sessions highlighted in gray). One factor was baseline versus postlearning and the other the 6-point time series over the 3-h recording. Five rats successfully learned the task within a single session and had a reliable postlearning LC recording. The behavioral performance of these rats is illustrated on Figure 4A. One rat was excluded because it did not express any odor-reward association learning (rat 1 in Table 1) and postlearning recordings for 2 rats did not satisfy the criteria for LC neurons (rats 2 and 3 in Table 1). ANOVA showed a significant interaction between the 2 factors (F_{5,20} = 5.02; P = 0.004). Subsequent Newman–Keuls test revealed a significant increase of LC activity at the 90- to 120-min time window after learning, compared with the same time period recorded during baseline (Fig. 4B). The LC activity during this time window was also significantly greater than during the neighboring 30-min time periods of the same session (Fig. 4B).

In a subsequent analysis, all 17 postlearning sessions (n = 7 rats) where reliable LC recording could be obtained were used. In some cases, this dataset included multiple sessions obtained from the same rat, which were recorded on different days, after different learning experience and with different LC cells (Table 1). A 2-way ANOVA for repeated measures revealed a significant interaction (F_{5,110} = 3.54; P = 0.005). Subsequent post hoc comparisons showed that the increase in firing rate of LC neurons during SWS at around 2 h after the learning experience was significant compared with the similar time window during baseline recording (Fig. 5A). This increase was also significantly different from the firing rate in the preceding time window. The postlearning LC awake firing rate is shown at the Figure 5B. Two-way ANOVA for repeated measures confirmed that there were no significant main effects and no significant interaction (F_{5,110} = 1.74, ns).

The SWS-associated increase in LC firing was not accompanied by major changes in cortical EEG, as illustrated in Figure 2A and the Supplementary Figure 2A-E. The power spectra of SWS episodes associated with low LC firing rate and the SWS episodes with increased LC activity of the same session reflected a typical SWS state with predominant delta frequency (Figs 2A and 6A). The delta power (amplitude) during all 13 postlearning recording sessions with detected LC activation did not differ from corresponding baseline sessions for the entire recording time (Fig. 6B). There was also no correlation between the LC rate and delta power when all SWS episodes of the 13 postlearning recording sessions with detected LC activation were considered (Fig. 6C). There was no such correlation during the baseline sessions (Supplementary Fig. 3). Finally, REM sleep was relatively sparse and associated LC firing was virtually absent in the baseline condition.

**Discussion**

The temporal firing pattern of the noradrenergic cells during SWS after stimulus–reinforcement contingency learning was strikingly different from that of several control conditions: nonmanipulated, randomly rewarded exploration in the training environment and procedural learning to dig (Figs 3–5). In all control conditions, there was a tendency for a gradual decline in the LC firing rate during SWS episodes over 3 h recording session (Figs 3–5). LC activity in trained rats showed
Figure 4. Behavioral performance (A) and the temporal dynamics of SWS-associated LC activity (B) during baseline (white bars) and postlearning (hatched bars) recording of rats that successfully learned the task within a single learning session (n = 5). The average latency of response of the first 2 (Begin) and the last 2 (End) trials are shown; **P < 0.05. LC firing rate during SWS is normalized to the corresponding awake rate of the same recording session. Note a gradual decrease in SWS firing rate over time during the baseline, and the significant, transient increase at 2 h after learning (**significantly different from corresponding time interval during the baseline P < 0.01; #significantly different from the other 30-min epochs of the same postlearning session, P < 0.05).

Figure 5. Firing rate of LC units during SWS (A) and awake (B) episodes occurring in successive 30-min epochs of a baseline recording (white bars, n = 7) and after a learning session (striped bars, n = 17). Note the general decrease in LC firing rate over time during both behavioral states. The significant, transient increase at 2 h after learning was observed during SWS episodes only (*significantly different from corresponding time interval during baseline P < 0.01; #significantly different from the previous 30-min epoch after learning, P < 0.01). LC firing rate is normalized to the average awake rate of the same recording session.

Figure 6. (A) Mean power spectra for postlearning sleep session. For each of the 13 recording sessions where the LC increase was significant, the power spectrum was averaged over the SWS episodes during the first hour of recording and then over the SWS episodes of period with elevated LC firing of the same recording session, as illustrated on Figure 2A (middle and right panels). Depicted are the mean ± SEM of the 13 recordings before the increase of LC activity (dashed line) and during the SWS episodes with elevated LC activity (solid line). Paired t-tests with Bonferroni correction revealed no significant differences at any frequency. (B) The power (amplitude) of delta waves (0-4 Hz, DeltaRMS) during SWS episodes occurring in successive 30-min epochs of a baseline recording (dashed line) and of the postlearning sessions with the significant LC activation (solid line). (C) Absence of correlation between LC firing rate and delta power averaged over each SWS episode of the postlearning sessions with the significant LC activation.
the same monotonic decrease until the second hour when a transient increase of LC activity to the level of the previous quiet awake state appeared in the majority of postlearning recordings, with no major change in cortical electrophysiological or behavioral sleep state (Fig. 6). Thus during SWS, under certain conditions, cortical state can be dissociated from LC activity. This novel finding would not be predicted from well documented fluctuations of LC rates over the sleep–awake cycle (Roussel et al. 1967; Hobson et al. 1975; Aston-Jones and Bloom 1981). There is evidence from the literature, however, that LC cells can be induced pharmacologically to increase their firing rate during both SWS and REM sleep without altering the forebrain EEG pattern or behavior associated with these states (Gervasoni et al. 1998). In that study, iontophoretic application of the gamma amino butyric acid (GABA) antagonist bicuculline into LC during SWS or REM sleep dramatically increased LC firing without waking the head-restrained unanesthetized rats. The release from GABA inhibition may well underlie the phenomenon described in the present study, although the origins of this inhibition remain to be established (Maloney et al. 1999; Verret et al. 2006).

The delayed SWS-associated increase in LC activity corroborates our earlier results showing increased release of NE in the frontal cortex 2 h after learning (Tronel et al. 2004), as well as selective susceptibility of memory to beta-noradrenergic antagonists during the same time window (Roulet and Sara 1998). Those results had left open the question of whether or not the delayed release of NE in the cortex actually corresponded to an increase of firing of LC cells. The present report clearly resolves this issue, but now leaves open the intriguing question concerning what drives the LC at this specific postlearning time window.

The real significance of the present results lies in the surprising fact that the delayed temporal window of LC excitation occurs exclusively during SWS, with no detectable change in cortical EEG. SWS, a state in which activity of LC neurons is normally diminished, is the preferred state in which a "replay" of activity of neuronal ensembles, active during learning, occurs. Off-line reactivation or replay of neuronal activity recorded from the hippocampus during previous behavioral episodes was first reported by Pavlides and Winson (1989). The subsequent development of ensemble recording techniques, permitting simultaneous recording of large populations of neurons revealed that cells that fired during awake behavior, tended to fire together during subsequent SWS (Wilson and Maunaghton 1994; Skaggs and McNaughton 1996); although one study reported replay during REM sleep, as well (Louie and Wilson 2001). This has now been shown also in neocortex and other brain regions (Qin et al. 1997; Pennartz et al. 2004) and is taken as neurophysiological evidence for the formation of cell assemblies to underlie memory, as proposed by Hebb (1949). Activation of the noradrenergic system during this replay, or later during replay-triggered synaptic plasticity processes, would promote the stability of these cell assemblies.

It is interesting to note that increase in LC activity during SWS occurs not only after new learning, but also after a retrieval or extinction session. This is in line with the studies that show replay after the rat retrieves a well-trained behavioral repertory, usually running laps on a familiar track. Both phenomena corroborate the notion that reactivated memories are in a labile state for some time, requiring a time-dependent "reconsolidation" process that in some ways recapitulates the initial consolidation process (see Sara 2000a, 2000b for reviews).

Kety's original prophesy suggested that NE should promote memory by selectively reinforcing neural networks that are activated during arousal or learning. We can extend this idea now to include memories that are reactivated off-line during SWS. Given the ubiquitous projections of LC neurons (Jones 1991), the increase in LC activity should release NE in key target regions where reactivation is taking place. If the timing is right, NE will serve to promote plasticity during replay of these networks. The temporal dynamics of the replay phenomenon is largely unknown. Most investigators report a relatively short-lived reactivation in the first hour after learning (but see Pennartz et al. 2004), but to our knowledge, there has been no investigation of longer postlearning time windows. It is quite possible that reactivation of these neural circuits occurs more than once, with the possibility of temporal contiguity with LC activation. Even without a close temporal relation between LC activity and network replay, NE released in cortex and hippocampus could act through volume transmission to initiate molecular cascades necessary for the formation of a long-term memory trace (Kety 1972; Bernabeu et al. 1997). Long-term memory consolidation would then be promoted by changes in gene expression. It has, indeed, been shown that changes in activity of neuromodulatory neurons over the sleep/wake cycle will result in changes in expression of specific genes and that the level of activity of LC neurons, in particular, is of crucial importance for expression of several immediate early genes associated with long-term plasticity (Tononi et al. 1995; Cirelli and Tononi 2004).

Although the cell ensembles replay data are compelling in terms of memory consolidation, there is, so far, no behavioral evidence that subsequent memory performance is in any way related to the network replay or that the off-line replay is necessary for retention. The same might be said for the present results. We have thus far not attempted to correlate LC activity during SWS with subsequent retention performance. The odor–reward association task was designed to lend itself to studies of memory consolidation, that is, to produce a robust, reliable memory trace with a minimal amount of training within a single session (Tronel and Sara 2002). Rats learn the task in a few trials and make almost no errors when tested a few days later, making it, indeed, impractical, if not impossible, to correlate the retention performance and LC activity. It takes about 3–4 weeks to show reliable retention deficits in this task; during which time there would be a risk of losing the implant and compromising histological analysis of the electrode placement. Nevertheless, it would be of great interest in future studies to evaluate the impact of the degree of LC activation subsequent to learning and long-term retention.

The here-reported discovery of the learning-dependent delayed LC activation opens new vistas for thinking about brain stem–cortical interaction related to off-line memory consolidation during sleep. To fully understand the functional role of this LC activation during sleep requires analyses on several levels, including assessment of gene activation specifically during the critical time window. Moreover, it is essential to investigate the temporal relationship between reactivation of network ensembles in forebrain and LC unit activity through simultaneous recordings. Such an approach will tell us whether the LC is actually a part of the network being replayed off-line during SWS. This is a distinct possibility because we have
already seen a strong task-related activation of LC neurons during learning often in tandem with cortical unit activity (Bouret and Sara 2004, 2005). It is an intriguing possibility that the hippocampal-cortical replay activity during SWS might act to "wake-up" the LC, with consequent long-lasting reinforcement of the reactivated networks.

Supplementary Material

Supplementary material can be found at: [http://www.cercor.oxfordjournals.org/](http://www.cercor.oxfordjournals.org/)

Funding

Volkswagenstiftung; and CNRS UMR 7102.

Notes

Technical assistance was provided by Yves Moricard and Jacques Fuzellier. Thanks to Sebastien Bouret for comments on the manuscript.

Conflict of Interest

None declared.

Address correspondence to Dr. Susan J. Sara, LPPA, CNRS, UMR 7252, Collège de France, 75005, Paris, France. Email: sjsara@ccr.jussieu.fr.

References


Björvatn B, Fagerland S, Ursin R. 1998. EEG power densities (0.5-20 Hz) in different sleep-wake stages in rats. Physiol Behav. 63:413-417.


