Level of Arousal during the Small Irregular Activity State in the Rat Hippocampal EEG

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Running title: Level of arousal during SIA

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ABSTRACT. The sleeping rat cycles between two well-characterized hippocampal physiological states, large irregular activity (LIA) during slow-wave sleep (SWS) and theta activity during rapid eye-movement sleep (REM). A third, less well-characterized EEG state, termed “small irregular activity” (SIA), has been reported to occur when an animal is startled out of sleep without moving, and during active waking when it abruptly freezes. We recently found that the hippocampal population activity of a spontaneous sleep state whose EEG resembles SIA reflects the rat’s current location in space, suggesting that it is also a state of heightened arousal. To test whether this spontaneous SIA state corresponds to the SIA state reported in the literature, and to compare the level of arousal during SIA to the other well-characterized physiological states, we recorded unit activity from ensembles of hippocampal CA1 pyramidal cells, EEG from the hippocampus and the neocortex, and EMG from the dorsal neck musculature in rats presented with auditory stimuli while foraging for randomly-scattered food pellets and while sleeping. Auditory stimuli presented during sleep reliably induced SIA episodes very similar to spontaneous SIA in hippocampal and neocortical EEG amplitudes and power spectra, EMG amplitude, and CA1 population activity. Both spontaneous and elicited SIA exhibited neocortical desynchronization, and both had EMG amplitude comparable to that of waking LIA. We conclude based on this and other evidence that spontaneous SIA and elicited SIA correspond to a single state, and that the level of arousal in SIA is higher than in the well-characterized sleep states, but lower than the active theta state.

Key words: sleep, ensemble, place cells, theta, LIA
INTRODUCTION

Three physiological states have been recognized in the rat hippocampus, generally known as the theta, large irregular activity (LIA), and small irregular activity (SIA) states, after the type of EEG associated with each. The properties of these hippocampal states and their relationship to global physiological states are summarized in Table 1. The literature on theta and LIA in the rat is quite extensive; in contrast, the literature on SIA is relatively scarce. Perhaps the main reason for this is that when EEG alone is examined, SIA appears, mainly during sleep, in frequent but brief (~2 sec) flattenings of the voltage trace. Given the overall complexity of the EEG, the importance of these events is not obvious. However, when rasters of substantial numbers of simultaneously recorded CA1 pyramidal cells are added to the picture, the distinctiveness of the SIA state becomes very clear (Skaggs 1995; Jarosiewicz and Skaggs 1999, 2001; Jarosiewicz et al. 2002; see also Fig. 1 of the current paper). SIA is characterized by a sparse pattern of population activity, in which a small subset (~3-5%) of pyramidal cells shows continuous activity while the rest are nearly silent. Moreover, the same group of cells is usually active across long sequences of SIA episodes. Jarosiewicz et al. (2002) showed that the SIA-active cells largely correspond to place cells whose place fields encompass the location where the rat sleeps, suggesting that SIA might be a state of increased arousal.

The literature provides some additional support for such a suggestion. Pickenhain and Klingberg (1967) reported a “low-amplitude irregular activity” in the hippocampus of rats in response to novel or unfamiliar stimuli when no orienting movements are made; e.g., when a click awakens them from sleep. Vanderwolf (1971) and Whishaw (1972) reported a similar suppression of hippocampal activity, which they called SIA, when rats suddenly arrest voluntary movement or change from a resting or sleeping state to an alert state, as indicated by neocortical desynchronization, without moving. Other groups of researchers have reported similar EEG states during sleep: Roldán et al (1963) observed “arousal-like periods” of EEG desynchronization in both neocortex and hippocampus at the end of REM and sometimes during SWS, similar to the state they observed when the rat is startled out of sleep. Bergmann
et al. (1987) reported the existence of “low-amplitude sleep,” characterized by low hippocampal and cortical EEG amplitude and low EMG amplitude, similar to a state they observed when the rat is startled while awake. Both “arousal-like periods” and “low-amplitude sleep” probably correspond to Vanderwolf’s SIA; we have chosen to adopt Vanderwolf’s terminology.

The aim of the current study was to determine whether spontaneous and arousal-elicited SIA correspond to a single physiological state, and to characterize the level of arousal during SIA. There is no consensus in the literature on an absolute definition of arousal level, but EMG amplitude and neocortical EEG are commonly used heuristic measures: EMG amplitude is higher in the well-characterized waking states than the well-characterized sleep states (Gottesmann 1992), and neocortical desynchronization is often interpreted as an indication of arousal (Pravdich-Neminsky 1913; Berger 1929; Moruzzi and Magoun 1949; Green and Arduini 1954; Pickenhain and Klingberg 1967; O’Keefe and Nadel 1978; Vanderwolf 1969; Whishaw 1972; Gottesmann 1992). Thus, we recorded and characterized the neocortical EEG and EMG, in addition to hippocampal EEG and CA1 ensemble activity, of spontaneous SIA and of SIA elicited by auditory stimuli during sleep and waking, and compared them to each other and to the other well-characterized sleep and waking states.

MATERIALS AND METHODS

Subjects. Data were collected from 8 male Sprague Dawley rats, weighing between 350 and 500 gm at the time of surgery. Each rat was housed individually in a 12 hr light/dark cycle in a temperature-controlled room with food and water available ad libitum. For 1-2 weeks before surgery, each rat was handled and gradually accustomed to the recording room environment for several hours a day, and food-deprived to about 95% of its ad libitum weight to motivate it to forage for randomly scattered sweetened food pellets so that recordings could be tracked between sleep and waking behavior. Recordings were made during the light phase of the cycle.
Behavioral Apparatus. Recordings were performed while rats slept or foraged for randomly scattered food pellets. Both behaviors took place on a circular vinyl-covered platform arena (~1.5 m dia.) with a 40 cm tall transparent border around the edge. The arena was located inside a sound-attenuated room with visual cues on the walls, and a computer speaker was placed underneath the arena for administering auditory stimuli. To prevent habituation, variable auditory stimuli were chosen from a set of 102 different computer game and Microsoft Windows OS audio files, varying in duration (1-2 sec) and amplitudes, and each played only once for a given rat. The speaker volume was generally held constant across rats, but if a on any given recording day a rat was repeatedly awakened by the stimuli to the point of walking around, the volume was turned down and the sleep session was restarted. During active foraging (“run”) sessions, the volume was set higher but still within a range comfortable to the human ear; the rats rarely showed any responses to these stimuli. Stimuli were played at pseudorandom intervals averaging 2 minutes apart, typically yielding 15 stimuli per sleep or run session. Stimuli during the sleep session were postponed as necessary to ensure they occurred following at least 10 seconds of continuous sleep (SWS or REM). The onsets and offsets of these stimuli were automatically flagged by the data acquisition software. We found that computer-generated stimuli were ineffective in causing rats to freeze during the run session, so in later rats, we manually generated auditory/visual stimuli that were known to make rats freeze. These included tearing a piece of paper, opening an umbrella, crinkling a can, nudging the wastebasket along the floor, dropping a pen, etc. These events were flagged manually, and thus not as precisely as the computer-generated auditory stimuli.

Surgery. All surgery was performed under sterile conditions. Rats were anesthetized with ketamine (60 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.), and boosts of ketamine and xylazine were given during surgery as necessary. Once deeply anesthetized, the rats were secured in earbars in a Kopf stereotaxic frame (David Kopf Instruments, Tujunga, CA). A small (~1 cm) incision was made along the midline of the scalp to expose the cranium. The skin and connective tissue were retracted, and seven small holes were
drilled into the cranium to accommodate jeweler’s screws, one of which was later connected to a ground channel.

A small hole was drilled over the frontal cortex (~1 mm dia., centered on 2 mm anterior, 2 mm lateral from bregma) to accommodate a bipolar neocortical EEG recording electrode, consisting of a twisted pair of 0.0045” (coated) stainless steel wires with ends spaced 2 mm apart vertically. The EMG electrode, consisting of a twisted pair of 0.0045” (coated) stainless steel wires, each with 1mm exposed at the tip and bent 2 mm back to form a hook, was inserted into the dorsal neck musculature by routing it under the skin from the incision. A few square 10 mA pulses of 1 ms duration were passed through the EMG electrodes at 1 Hz using a stimulus isolation unit and a Grass S88 stimulator to check for muscle twitch, to ensure proper placement of the wires. Another larger hole was drilled over the right hippocampus (~2 mm diameter, centered on 3.5 mm posterior, 2-3 mm lateral from bregma). The dura was retracted, and the exposed cortex was covered with sterilized petroleum jelly. The base of a "hyperdrive," which contained 12 individually drivable tetrodes and two single-channel reference/EEG electrodes all bundled to ~1.5 mm diameter at the base, was lowered toward the exposed cortex. In addition, a small hole was drilled at 0.5 mm anterior, 4.0 mm lateral from bregma to accommodate a 26 gauge guide cannula (Plastics One, Roanoke, VA), which entered the brain at a 30 degree angle ML, its tip inserted to within 1 mm of the medial septum/diagonal band of Broca. These cannulae were used for microinfusion studies not reported in this paper; no animal received infusions before or during data collection for the studies reported here. All implants were cemented in place with dental acrylic, which was anchored to the cranium by jeweler's screws.

Just after surgery, the tetrodes and reference electrodes were lowered ~680 µm toward the hippocampus, and the wound was covered with antibiotic ointment and a mild local anesthetic ointment. Over the next few days, the wound was cleaned and ointment was reapplied daily until the animal recovered. Tetrodes were gradually lowered over a few hours each day until they arrived at the hippocampal CA1 pyramidal cell layer (~2 mm deep), which was identified by its well-characterized EEG

Electrophysiology and recording. For data acquisition, the top of the hyperdrive was connected to a headstage containing preamplifiers and a ring of light-emitting diodes used for position tracking by a camera mounted on the ceiling over the recording chamber. The headstage was attached to a pair of soft, flexible cables, partially suspended by a counterweight system to help ease the load on the rat's head. The cables ascended through the ceiling of the recording chamber into the adjoining room, where they connected to the Cheetah recording system (Neuralynx, Tucson, AZ), consisting of eight 8 channel amplifiers with software-configurable high- and low-pass filters, feeding their output to a custom-made controller and analog-to-digital processor. During recording, signals from each channel of each tetrode were filtered to 600-6000 Hz, sampled at 32 kHz per channel, formatted, and fed to a Windows NT system (Neuralynx, Inc., Tucson, AZ) running custom-written acquisition and control software. Each time the signal on any one of the tetrode channels crossed a specified threshold, a 1 msec sample of data from all four channels of that tetrode was written to disk, beginning 0.25 msec before the threshold was crossed, capturing the spike waveform on each channel along with its timestamp. Continuous recordings of EEG signals were also obtained from one channel on each tetrode, from an EEG electrode near the hippocampal fissure, from an EEG electrode in the prefrontal cortex, and from an EMG electrode in the dorsal neck musculature, at a bandwidth of 1-475 Hz (EEGs) or 100-475 Hz (EMG) and a sampling rate of 999 Hz. At the same time, position records containing information about the distribution of light across the video image were acquired at 60 Hz and written to disk. The rat's velocity was estimated as the change in position two timestamps before and two timestamps after the current timestamp, divided by the elapsed time. The error of the tracker is approximately one-half the width of the ring of light-emitting diodes on the headstage, or 2.5 cm.
Once an adequate number of stable CA1 complex-spike cells were obtained and robust theta activity was visible on the hippocampal EEG electrode during locomotion, a recording session was performed. EEG and EMG signals, spike waveforms, and the position of the rat were recorded simultaneously while the rat slept, ran for randomly scattered food pellets, or performed some sequential combination of the two. Approximately 10-30 recording sessions ("data sets"), each on separate days, were performed for each animal, until damage from the tetrodes made cells difficult to find or until the animal otherwise became unusable, at which point the animal was humanely killed and its hyperdrive was removed for reuse.

Cell isolation. Spike waveforms, EEG and EMG signals, and the rat's position data, along with their respective timestamps, were stored onto disk during the recording session for off-line analysis. Spikes were assigned to individual units by automated cluster-cutting software (Klustakwik, K. D. Harris), and clusters were then manually verified and cleaned using Mclust (A. D. Redish, current address, University of Minnesota, Minneapolis MN). Isolated units were then judged to be pyramidal cells or interneurons (Fox and Ranck 1981), or artifact, according to their average waveforms, autocorrelograms, interspike interval histograms, etc.; only those units judged to be relatively clean, well-isolated pyramidal cells were included in further analysis.

Sleep state delineation. Physiological states were delineated in 500 msec bins by a custom-written algorithm according to the following criteria:

- If total power (root-mean-square area under the curve) in the theta range (5-10 Hz) in the hippocampal EEG was at least 2 x the power in the LIA range (1-5 Hz), the bin was classified as “theta.”

- If LIA power was greater than theta power, it was classified as “LIA.” LIA from the sleep session was divided into waking LIA or SWS on the basis of neocortical EEG amplitude: LIA whose neocortical EEG amplitude fell below a set threshold (i.e. whose neocortical EEG was
desynchronized) was classified as “waking LIA,” and LIA whose neocortical EEG amplitude exceeded the threshold (i.e. showed large-amplitude slow waves) was classified as “SWS.” The threshold was set separately for each data set by inspection of the neocortical EEG amplitude distribution, which was typically bimodal with a large sharp peak at the low end (corresponding to desynchronization) and a small, wide peak at the high end (corresponding to large-amplitude slow waves); the threshold was set at the local minimum between these peaks.

- Theta whose EMG amplitude during the sleep session exceeded a set threshold was discarded as ambiguous; theta whose EMG amplitude during the sleep session fell below this threshold was classified as “REM”. The threshold was determined separately for each data set by inspection of the EMG amplitude distribution, which was typically bimodal with a large sharp peak at the low end (corresponding to sleep) and a small, wide peak at the high end (corresponding to waking); the threshold was set at the local minimum between these peaks. All theta during the run session was classified as “run.”

- If the hippocampal EEG amplitude was lower than a specified threshold, the bin was classified as “SIA”, regardless of its power spectrum. The threshold was determined separately for each data set by calculating the 20th percentile of the hippocampal EEG amplitude distribution from a continuous bout of sleep, since SIA was previously found to occupy approximately 20% of sleep (Jarosiewicz et al. 2002), and visually finding a local minimum near that amplitude in the distribution. All SIA beginning at stimulus onset and extending up to 10 seconds after was classified as “elicited SIA.” All SIA occurring outside of the 10 seconds following a stimulus was classified as “spontaneous SIA.” Although most elicited SIA episodes were shorter than 10 seconds, the 10-second time interval was chosen to minimize the misclassification of spontaneous SIA as elicited SIA. It was rare to observe multiple SIA episodes within a 10 second interval.

*Data analysis.* Except where otherwise indicated, statistics were computed on data set means, and figures show grand means and SEMs. To construct mean power spectra, the power spectral density estimate of
each 500 ms bin during sleep was calculated using Welch’s nonparametric averaged, modified periodogram method (Welch 1967), with a window size of 490 ms and a sampling frequency of 499.5 Hz. For each data set, bins were classified into physiological states as described above, and the mean power spectrum for a given state was the mean of the power spectra of the bins classified in that state. To compare power spectra across physiological states, mean powers of the specified frequency ranges were calculated for each data set, and statistics were done on these data set means. To quantify the similarity of power spectra in particular frequency ranges and EEG amplitudes between elicited and spontaneous SIA, linear regressions were done on the 8 data set means for elicited SIA and the 8 data set means for spontaneous SIA.

RESULTS

The structure of spontaneous and elicited SIA during sleep.

Auditory stimuli that were played during sleep reliably elicited SIA episodes that were visually indistinguishable from spontaneous SIA episodes (Fig. 1). In both spontaneous and elicited SIA, the hippocampal and neocortical EEG abruptly flattened, and most hippocampal complex-spike cells became quiet, except for a small subset of cells that abruptly became active at SIA onset and remained active through the duration of the SIA episode. These “SIA-active” cells were previously shown largely to be place cells whose place fields span the location in which the rat is sleeping (Jarosiewicz et al. 2002). The same cells were active during elicited SIA as during spontaneous SIA. The EMG amplitude of both elicited and spontaneous SIA remained as low as in the sleep period just before them, but longer SIA episodes were sometimes followed by movement, accompanied by an increase in muscle tone and the appearance of small-amplitude theta activity in the hippocampal EEG (Fig. 1A,B).

Effect of auditory stimuli on EEG and EMG amplitudes.

Figure 2 illustrates the relative consistency and robustness of the effect of auditory stimuli administered during sleep vs. during run. To construct this plot, the mean amplitudes of hippocampal EEG, neocortical EEG, and EMG, normalized by their mean amplitudes during SWS, were calculated for
each 500 ms bin around stimulus onset for each dataset, and the grand mean and SEM of the 8 data set means are plotted. When an auditory stimulus was played during sleep (SWS or REM), the hippocampal and neocortical EEG reliably abruptly flattened, and EMG amplitude sometimes increased after a few seconds when movement occurred. The mean amplitude of the hippocampal EEG in the period 1 to 5 seconds following stimulus onset (0.63 ± 0.03) was significantly reduced compared to the period 1 to 5 seconds prior to stimulus onset (1.00 ± 0.01; paired 1-tailed t-test; p < 0.00001). The mean amplitude of the neocortical EEG after stimulus onset (0.59 ± 0.05) was also significantly lower than the amplitude before stimulus onset (0.94 ± 0.03; p = 0.0003). The same auditory stimuli played during run, even at a louder volume, had no obvious effect on either behavior or physiology (based on 2 data sets). To test whether freezing behavior is critical for SIA, we caused the rat to freeze by various methods (e.g. tearing a piece of paper, etc.) and repeated the analysis. We still did not observe any robust SIA in response to these stimuli; a slight decrease in hippocampal EEG amplitude was sometimes observed, but never as dramatically as during sleep. Constructing the event-triggered average using only cases in which the rat displayed freezing responses (3 data sets), we found that the hippocampal EEG amplitude decreased slightly at stimulus onset (0.78 ± 0.03 before and 0.70 ± 0.03 after; p = 0.007), but much less than during sleep. The neocortical EEG amplitude did not change significantly, as it was already desynchronized during run. The EMG amplitude also did not change significantly. The transient increases at stimulus onset may be attributable in part to electrical artifact and/or volume-conducted field potentials from the neck EMG accompanying the occasional jerks of the head as the rat oriented to the stimulus. They might also correspond to the “evoked potentials” sometimes observed in the hippocampus and neocortex in response to sensory stimuli (Pickenhain and Klingberg 1965; Brankack and Buzsáki 1986; Deadwyler et al. 1981; Jirsa et al. 1992), or they may be a rat analogue of the evoked “K-complexes” observed in humans (Davis et al. 1939; Loomis et al. 1939; Roth et al. 1956; Ehrhart et al. 1981). Since we were unable to elicit robust SIA during run, no further analysis was done on the data in which stimuli were administered during run; all subsequent analysis was done on the 8 data sets in which stimuli were administered during sleep.
Comparison of EEG and EMG amplitudes across physiological states.

As described in Methods, sleep states were classified as “theta (REM),” “theta (run),” “waking LIA,” “SWS,” “SIA,” or “other” using an automated algorithm based on EEG and EMG characteristics. All SIA beginning at stimulus onset and extending up to 10 seconds after was classified as “elicited SIA”; the rest of SIA was classified as “spontaneous SIA.” The RMS amplitudes of hippocampal EEG, neocortical EEG, and EMG, normalized by their respective means during SWS, were calculated for elicited SIA, spontaneous SIA, waking LIA, SWS, REM, and run in each data set (Fig. 3A). To compare the mean amplitudes of neocortical EEG and EMG between elicited and spontaneous SIA, a linear regression was done on the mean amplitudes from each data set; a high correlation signifies that the mean amplitude of elicited SIA is similar to the mean amplitude of spontaneous SIA in each data set. The correlation between the mean neocortical EEG amplitudes of elicited SIA (0.47 ± 0.03) and spontaneous SIA (0.49 ± 0.04) was 0.70 (Fig. 3B), and the correlation between the mean EMG amplitudes of elicited SIA (1.23 ± 0.12) and spontaneous SIA (1.30 ± 0.12) was 0.89 (Fig. 3C). Thus, elicited and spontaneous SIA have similar neocortical EEG and EMG amplitudes.

Both elicited and spontaneous SIA had a significantly lower mean neocortical EEG amplitude than SWS (1-tailed paired t-test; p < 0.00001 for both elicited and spontaneous SIA) and REM (p < 0.00001 and p = 0.0001, respectively). The fact that the neocortical EEG amplitude during REM was not as low as during run is attributable to the fact that our sleep state delineation only considered hippocampal EEG; intermediate sleep (Gottesmann 1973, 1992), the period of transition from SWS to REM during which the neocortical EEG still exhibits large-amplitude slow waves but theta activity already appears in the hippocampus, was grouped with REM in this study. Both elicited and spontaneous SIA had significantly lower EMG amplitude than run (p = 0.008 and 0.009, respectively), but slightly higher amplitude than REM (p = 0.04 and 0.02) and SWS (p = 0.05 and 0.02). They were similar in EMG amplitude to waking LIA. Thus, the neocortical EEG of SIA is desynchronized, and the EMG amplitude is higher than in sleep but lower than in run, and comparable to that of waking LIA.
Comparison of hippocampal and neocortical EEG power spectra across physiological states.

To compare physiological states to one another in more detail, mean power spectra were constructed of hippocampal EEG (Fig. 4A,B) and neocortical EEG (Fig. 4C,D) for elicited and spontaneous SIA, waking LIA, SWS, REM, and run. By definition, in the hippocampal EEG power spectrum, REM and run have high power at theta frequency (5-10 Hz), SWS and waking LIA have high power between 1-5 Hz, and elicited and spontaneous SIA have low total power. The shapes of the power spectra of elicited and spontaneous SIA, however, were not predefined. They were found to have almost identical grand mean hippocampal power spectra (r = 0.999), with comparable levels of total power in both the low frequencies (1-10 Hz; regression across data sets: r = 0.998) and the high frequencies (80-160 Hz, chosen to exclude the artifact at 60 and 180 Hz; regression across datasets: r = 0.999).

There were significant differences across physiological states in the mean power in the low frequency range (two-way ANOVA with 5 and 7 df; F = 30.7; p < 10^{-11}) and the high frequency range (F = 7.67; p = 0.00006); post-hoc paired t-tests revealed that both elicited and spontaneous SIA had significantly less mean power than any of the other physiological states in both the low frequency range (for elicited SIA vs. waking LIA, SWS, REM, and run: p = 0.0001, 0.0001, 0.0005, and 0.0001, respectively; for spontaneous SIA vs. waking LIA, SWS, REM, and run: p = 0.0001, 0.0001, 0.0006, and 0.0001, respectively) and the high frequency range (for elicited SIA: p = 0.025, 0.015, 0.017, and 0.0008; for spontaneous SIA: p = 0.028, 0.016, 0.018, and 0.0006). Both elicited and spontaneous SIA were also found to have a small peak in the low-frequency (type 2) theta range (mean across data sets = 6.21 ± 0.20 and 6.12 ± 0.16 Hz, respectively), which is significantly lower than the peak frequency of theta during run (8.10 ± 0.08 Hz; p < 0.00001 for both elicited and spontaneous SIA) and REM (7.34 ± 0.07 Hz; p < 0.00001 for both elicited and spontaneous SIA). Thus, the power spectra of the hippocampal EEG are quite similar between spontaneous and elicited SIA.

The grand mean neocortical EEG power spectra of elicited and spontaneous SIA were also highly correlated (r = 0.98). Spontaneous SIA appeared slightly higher in amplitude in low frequencies (1-10 Hz) than elicited SIA, but this difference varied across data sets and was not significant. It is probably
attributable to the sleep state delineation method, which was more likely to allow 500 msec segments classified as spontaneous SIA than 500 msec segments classified as elicited SIA to contain small amounts of SWS, because auditory stimuli almost always elicited SIA (see Figure 2, left column). Interestingly, the power spectra also revealed that the neocortical desynchronization present during run differed from the neocortical desynchronization present during SIA; run had significantly higher power in the high frequencies (80-160 Hz) than elicited SIA and spontaneous SIA (p = 0.00006 and 0.0002, respectively). It also has significantly higher power in this range than waking LIA (p = 0.0009) and REM (p = 0.0004), whose neocortical EEG are also desynchronized. This unexpected difference between the desynchronization present during run on the one hand and SIA, waking LIA, and REM on the other hand was present in all 8 rats.

Comparison of hippocampal unit activity between elicited and spontaneous SIA.

To quantify the similarity of the CA1 ensemble spike activity between elicited and spontaneous SIA, the mean firing rate of each cell from each data set (total = 383 cells) was calculated for elicited SIA and for spontaneous SIA. Those cells that were active in spontaneous SIA were also found to be active in elicited SIA (Fig. 5A). Furthermore, the cells that were not very active in SIA (mean < ~1 Hz; about 95% of cells) still had similar mean firing rates in elicited and spontaneous SIA (Fig. 5B). The correlation between the population activity in elicited and spontaneous SIA when all cells were taken together was 0.975. The mean and SEM of each data set’s population activity correlation coefficient was 0.88 ± 0.04. The very similar population activity between elicited and spontaneous SIA provides further strong evidence that they correspond to a single physiological state.

DISCUSSION

Jarosiewicz et al. (2002) showed that SIA occurs frequently during sleep in the rat, that the hippocampal CA1 pyramidal cell ensemble activity during SIA is sparse, and that the pyramidal cells that are active
during SIA are place cells whose place fields encompass the location in which the rat is sleeping. In contrast, during REM and LIA, the population activity is not patently related to the current location of the rat. In REM, the temporal structure of hippocampal ensemble activity, like the hippocampal EEG, appears similar to the awake theta state (Skaggs and McNaughton 1998; Louie and Wilson 2001): individual complex spike cells are silent most of the time, but occasionally fire in bursts a few seconds long. Their temporal patterns statistically resemble the patterns of activity in the preceding waking pattern more than expected by chance, as though the hippocampus is replaying memories of the rat’s trajectories in the preceding waking period (Louie and Wilson 2001). In LIA, place selectivity is highly degraded (Foster et al. 1989; Kubie et al. 1985) or absent (O’Keefe and Nadel 1978; Best and Ranck 1982); the population of hippocampal CA1 cells has a diffuse pattern of activity, with transient increases during sharp waves (Buzsáki et al. 1992). The patterns of activity during LIA, particularly during sharp waves, also statistically resemble the patterns of activity during the preceding waking period more than expected by chance, but unlike the reactivation during REM, the reactivation during LIA is highly compressed in time (Pavlides and Winson 1989; Wilson and McNaughton 1994; Skaggs and McNaughton 1996; Nádasdy et al. 1999). The finding that the hippocampal ensemble activity during SIA reflects the rat’s current location in space (Jarosiewicz et al. 2002) raised the possibility that SIA is a state of heightened arousal relative to the other sleep states. Thus, to compare the level of arousal during this spontaneous SIA state with the other well-characterized physiological states, and to test whether spontaneous SIA corresponds to the SIA elicited by auditory stimuli (Pickenhain and Klingberg 1967; Vanderwolf 1971; Whishaw 1972; Roldán et al. 1963; Bergmann et al. 1987), the present study simultaneously recorded hippocampal and neocortical EEG, neck EMG, and hippocampal ensemble activity from behaving rats presented with auditory stimuli.

Results showed that (1) auditory stimuli presented during sleep reliably elicited SIA episodes very similar to spontaneous SIA episodes in EEG amplitude and power spectra, EMG amplitude, and hippocampal CA1 population activity; (2) the EMG amplitude, a measure of muscle tonus and therefore commonly used to judge behavioral arousal level, is significantly lower in elicited and spontaneous SIA
than in run, slightly but significantly higher than in REM and SWS, and comparable to waking LIA; and (3) the neocortical EEG, which is commonly used to judge the level of cognitive arousal, is desynchronized in spontaneous and elicited SIA. Thus, what we call “spontaneous” SIA is likely to also be elicited by a stimulus, but one that is uncontrolled or unobserved by the experimenter. This appears to be the case in the probable human analogue of SIA, “micro-arousals,” which occur spontaneously during sleep but can also be elicited by auditory stimuli (Schieber et al. 1968, 1971; Ehrhart and Muzet 1974; Halász et al. 1979; for review, see Terzano et al. 1991).

The finding that the hippocampal EEG during SIA has a small peak in the low frequency theta range suggests a possible relationship between SIA and “type 2” theta (also called “atropine-sensitive,” “sensory-elicited”, or “immobility” theta), which has a lower frequency than the typical movement-associated “type 1” theta, is present under urethane or ether anesthesia, and has a different pharmacological and laminar profile than type 1 theta (Kramis et al. 1975; Bland 1986). Type 2 theta is rarely observed in awake behaving rats, but is common in rabbits and guinea pigs, in whom it can readily be elicited by auditory stimuli during immobility (Kramis et al. 1975; Sainsbury and Montoya 1984; for review, see Bland 1986). The fact that SIA can be elicited similarly, and that it has a small power peak in the low-frequency theta range, suggests that SIA might simply be the rat analogue of type 2 theta. However, robust type 2 theta, comparable in amplitude to LIA, is present in urethane-anesthetized rats at the same recording site as baseline SIA (unpublished observations), and robust type 2 theta can be elicited by auditory stimuli in immobile rats in the presence of cats or ferrets (Sainsbury et al. 1987). Thus, either SIA is not simply the rat analogue of type 2 theta, or the amplitude of type 2 theta can vary significantly in different circumstances. Some studies report a positive correlation between theta frequency and/or amplitude and movement speed (Whishaw and Vanderwolf 1973; McFarland et al. 1975; Rivas et al. 1996; Slawinska and Kasicki 1998; but see Shin and Talnov 2001); thus, another possibility is that the low frequency, low amplitude theta visible in the SIA power spectrum is a low-amplitude, low-frequency type 1 theta emerging at the end of longer SIA episodes when the rat makes small movements.
Examination of the neocortical EEG power spectra unexpectedly reveals that run has more power across the high frequency range than SIA. This finding has several interesting implications, among them that what appears to be “desynchronization” in the raw EEG is not actually a homogeneous state, and thus, that its use as a measure of cognitive arousal should be used with discretion. In fact, run also has higher power across the high frequencies than waking LIA and REM, which also exhibit desynchronized neocortical EEG (although in this study, REM also included periods of intermediate sleep, so its desynchronization was not apparent in the graphs). Thus, it is possible that the power in the high frequency range of the neocortical EEG increases during run because the rat is actively moving around and/or engaged in associated behaviors such as whisking. Evidence for the latter comes from the finding that stimulating whiskers in an anesthetized rat produces an increase in high frequency oscillations (> 200 Hz) in the EEG of the barrel cortex (Jones and Barth 1999). If the presence or absence of movement is really the sole determinant of the power in the high frequencies of the neocortical EEG, then the difference in desynchronization between SIA and active waking is trivial.

Despite our efforts, we were unable to elicit robust SIA during active waking. One possible explanation is that inducing freezing by external stimuli is somehow not adequate to produce SIA during waking; it might be necessary for the freezing to be somehow initiated by the rat without influence from external cues. Another possibility is that waking SIA is simply much less robust than SIA occurring during sleep. Indeed, upon closer inspection of the two articles in which SIA was described to occur in rats that abruptly suppress ongoing movement (Bergmann et al. 1987; Vanderwolf 1971; Whishaw 1972), we found that the only examples shown of SIA in rats were in response to auditory stimuli administered during sleep; the two examples of SIA during waking (both in Whishaw 1972) were actually taken from a Mongolian gerbil. Thus, it is likely that the SIA that occurs in rats during waking is simply not as robust as it is during sleep, or as it is in other species. It is also possible that differences exist between strains of rats; Bergmann et al. (1987) used Sprague-Dawley rats like we did but Vanderwolf (1971) and Whishaw (1972) used hooded rats.
In summary, the present findings that elicited SIA so strongly resembles spontaneous SIA and that the neocortical EEG is desynchronized during SIA, taken with the previous finding that the hippocampal population activity during SIA reflects the rat’s awareness of its current location in space (Jarosiewicz et al. 2002), provide compelling evidence that the rat’s arousal level during SIA is heightened relative to the other well-characterized sleep states. However, its low EMG amplitude provides evidence that its level of arousal is not as high as in active waking. Further support for the latter comes from unpublished observations in our laboratory that the population activity in SIA reflects a memory for the location in which the rat fell asleep, rather than an assessment of the rat’s current location based on current visual information: when the rat is moved to a new location while asleep, the population activity during subsequent SIA episodes continues to reflect the rat’s old location in the room, although the rat’s spatial representation realigns to room coordinates in subsequent active waking periods, suggesting that the rat’s level of awareness and/or depth of processing of current visual input is not as high during SIA as during active waking. We conclude that the level of arousal during SIA lies somewhere between the awake LIA state and the awake theta state, sharing features with both.
ACKNOWLEDGEMENTS

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**FIGURE LEGENDS**

Figure 1. Spontaneous and elicited SIA. Each panel contains a 20 second sample of simultaneously-recorded hippocampal EEG (Hipp), neocortical EEG (Ctx), EMG recorded from the dorsal neck musculature (EMG), spikes from ensembles of simultaneously-recorded CA1 pyramidal cells (Units), and the rat’s velocity (vel, with zero aligned at the bottom of the panel) from a single sleep epoch. The tick marks at the top of panels B and D represent auditory stimulus onset and offset (Stim). A, Spontaneous SIA episode. The hippocampal and neocortical EEG abruptly flattened, and most hippocampal CA1 pyramidal cells became quiet, except for a small subset of cells whose place fields were in the current location of the rat. B, Example of an SIA episode elicited by an auditory stimulus from the same sleep session as A. Again, the neocortical and hippocampal EEGs flattened, and the same cells were active as during spontaneous SIA episodes. C, D, Examples of spontaneous (C) and elicited (D) SIA episodes from a different rat. Again, elicited SIA strongly resembled spontaneous SIA in hippocampal and neocortical EEG and population activity. SIA episodes usually evolved back into LIA, as in C and D; in these cases, the EMG remained the same amplitude as the preceding sleep period. Longer episodes, such as those in A and B, were sometimes followed by small movements or posture changes, accompanied by an increase in muscle tone and the appearance of small-amplitude theta activity in the hippocampal EEG.

Figure 2. Effect of auditory stimuli on EEG and EMG amplitude. The event-triggered average of the amplitudes of hippocampal EEG, neocortical EEG, and EMG are plotted around stimulus onset, after normalizing by their means during SWS. Bin size = 500 msec. When the auditory stimulus was played during sleep, the hippocampal and neocortical EEG abruptly flattened, and EMG amplitude often increased after a few seconds. The same auditory stimuli played during run, even at a louder volume, had no obvious effect on behavior or physiology. When sounds were generated manually that caused the rat to freeze, the hippocampal EEG amplitude decreased slightly, although much less consistently and robustly than during sleep. The neocortical EEG amplitude did not change significantly, as it was already desynchronized during run. The EMG amplitude also did not change significantly. The transient increases at stimulus onset are probably attributable to electrical artifact accompanying the occasional jerks of the head as the rat orients to the auditory stimulus, evoked potentials, and/or K-complexes.

Figure 3. Comparison of hippocampal and neocortical EEG and EMG amplitudes. The root-mean-square amplitude of hippocampal EEG, neocortical EEG, and EMG was calculated for elicited SIA (“eSIA”), spontaneous SIA (“sSIA”), waking LIA (“wLIA”), SWS, REM, and run in each data set. (A) Elicited SIA was comparable to spontaneous SIA in hippocampal EEG (by definition), neocortical EEG, and EMG amplitudes. The neocortical EEG amplitude of both eSIA and sSIA was lower than SWS and REM, and the EMG amplitude was comparable to that of waking LIA. Each data set’s elicited SIA was similar to its spontaneous SIA (B) in mean neocortical EEG amplitude ($r = 0.70$) and (C) in mean EMG amplitude ($r = 0.89$).

Figure 4. Comparison of hippocampal and neocortical EEG power spectra. Power spectra were constructed using Welch’s averaged, modified periodogram method (Welch 1967), with a window size of 490 ms and a sampling frequency of 499.5 Hz. eSIA = elicited SIA; sSIA = spontaneous SIA; wLIA = waking LIA; SWS = slow-wave sleep; REM = rapid eye movement sleep; run = active waking. A, B, By definition, REM and run have peaks at theta frequency (5-10 Hz) in the hippocampal EEG, and SWS and waking LIA have a peak between 1-5 Hz. Elicited and spontaneous SIA had almost identical hippocampal power spectra ($r = 0.999$); both had low total power across the frequency spectrum and a small peak in the low-frequency (type 2) theta range (mean across data sets ≈ 6.2 and 6.1 Hz, respectively). B, The same data as in A are plotted against log power to reveal detail in the high frequencies. C, D, Elicited and spontaneous SIA were also similar in neocortical EEG ($r = 0.98$). Spontaneous SIA appeared slightly
higher in amplitude in low frequencies (1 – 10 Hz) than elicited SIA, but this difference was not significant; it was probably attributable to the sleep state delineation method, which was more likely to allow SWS to contaminate segments classified as spontaneous SIA than elicited SIA (i.e. it was unlikely for SWS to occur within the 10 seconds following an auditory stimulus). Interestingly, the neocortical “desynchronization” present during SIA was different from that of run; eSIA and sSIA had significantly lower power in the high frequencies (80-160 Hz) than run (p = 0.00006 and p = 0.0002, respectively). The sharp peaks at 60, 180, and 200 Hz are attributable to artifact.

Figure 5. Hippocampal population activity during elicited vs. spontaneous SIA. Each cell’s mean firing rate in elicited vs. spontaneous SIA is plotted for all cells from all data sets (total = 383 cells). A, Standard plot, showing that cells that were active in spontaneous SIA were also active in elicited SIA. B, Log-log plot, revealing that a linear relationship also existed for the firing rates of cells that were relatively quiet (< ~1 Hz) during elicited and spontaneous SIA. The correlation across all cells taken together was 0.98. The mean ± SEM correlation coefficient for each data set was 0.88 ± 0.04.
<table>
<thead>
<tr>
<th>State</th>
<th>Hippocampal EEG</th>
<th>Neocortical EEG</th>
<th>Hippocampal population activity</th>
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</thead>
<tbody>
<tr>
<td>Active waking(^i)</td>
<td>Theta</td>
<td>Desynchronized</td>
<td>Place-related</td>
</tr>
<tr>
<td>Quiet waking(^{ii})</td>
<td>LIA</td>
<td>Desynchronized</td>
<td>Place-related activity strongly degraded; mostly diffuse, with increases in activity during sharp waves</td>
</tr>
<tr>
<td>Slow-wave sleep(^{iii})</td>
<td>LIA</td>
<td>Slow waves, spindles</td>
<td>Diffuse, with increases in activity during sharp waves</td>
</tr>
<tr>
<td>Intermediate sleep(^{iv})</td>
<td>Theta</td>
<td>Slow waves, spindles</td>
<td>Diffuse, begins to resemble active waking</td>
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<tr>
<td>REM(^v)</td>
<td>Theta</td>
<td>Desynchronized</td>
<td>Resembles active waking</td>
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<tr>
<td>SIA(^{vi})</td>
<td>SIA</td>
<td>Desynchronized</td>
<td>Place-related</td>
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For reviews, see O’Keefe and Nadel (1978); Gottesmann (1992); Skaggs and McNaughton (1998).


\(^{iii}\) Buzsáki et al. 1986, 1992; Gottesmann 1964; Steriade et al.,1993; McCormick and Bal 1997; Siapas and Wilson 1998.

\(^{iv}\) EEGs: Gottesmann 1973; Hippocampal population activity: B. Jarosiewicz, unpublished observations.

\(^{v}\) Rapid eye movement sleep, corresponding in humans to dream sleep (Dement and Kleitman 1957a,b); Louie and Wilson 2001.

Figure 1
Figure 3
Figure 4