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NREM Sleep with Low-Voltage EEG in the Rat

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Summary: NREM sleep in the rat has traditionally been defined by electroencephalographic (EEG) amplitudes above those of wakefulness (W) and paradoxical sleep (PS); we refer to this high-amplitude NREM sleep as "HS." We have found that ~5% of total time is occupied by episodes in which (a) EEG amplitude is low, distinguishing it from HS; (b) theta amplitude is low, distinguishing it from PS; and (c) electromyographic (EMG) amplitude is low, distinguishing it from W. We have called these low-EEG, low-theta, low-EMG episodes "low-amplitude sleep" (LS). Three studies are done to elucidate additional characteristics of LS. (a) Polygraphically scored 30-s epochs were matched with independent classifications of rat behavior as W, NREM, or PS; 87% of polygraphically scored LS epochs were matched with NREM sleep behavior. (b) Response thresholds to noxious stimuli were lowest in W, intermediate and similar in LS and HS, and highest in PS. (c) The incidence of PGO-type (ponto-geniculo-occipital) waves in W, HS, and LS were all very low in comparison with rates in PS. Thus, LS and HS exhibited similarly quiescent spontaneous behavior, similar intermediate response thresholds, and similar low rates of PGO-type activity. Accordingly, we have proposed that LS, along with HS, is an NREM sleep stage. **Key Words:** Sleep—Sleep stages in rats—Sleep scoring in rats—Sleep behavior in rats—Ponto-geniculo-occipital (PGO) spikes in rats.

Polygraphic recordings of the rat are usually divided into three stages. Waking (W) is defined by low-amplitude electroencephalogram (EEG), low-amplitude theta (recorded near the midline of the cortex), and high-amplitude electromyogram (EMG). High-amplitude sleep (HS)[1] is defined by high-amplitude irregular EEG waves at both lateral and midline sites and low-amplitude EMG. Paradoxical sleep (PS) is defined by low-amplitude lateral EEG, high-amplitude, fairly continuous theta (midline) activity, and low-amplitude EMG. Examples of W, HS, and PS are shown in Fig. 1. Inspection of

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[1] We use the term "high-amplitude sleep" (HS) rather than the popular term "slow wave sleep" because we score this state by amplitude alone. In fact, most scoring of this stage in the rat is similarly based upon EEG amplitude, since frequency is rarely measured and cannot be resolved in the slow paper speed recordings typically used. Borbély and Neuhaus (1) found that only a small percentage of rat NREM sleep contained substantial slow wave activity (positive zero crossing rate of <40/10 s). Also, the designation of all high-amplitude rat sleep as "slow wave sleep" may obscure functionally meaningful episodes of genuine EEG slowing that are independent of amplitude (2).

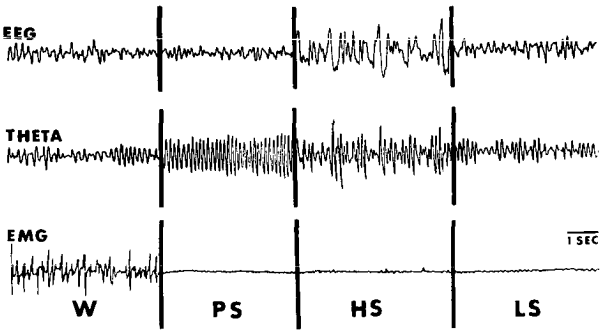

FIG. 1. Polygraphic recordings of electroencephalographic (EEG), theta, and electromyographic (EMG) activity during wakefulness (W), paradoxical sleep (PS), high-amplitude sleep (HS), and low-amplitude sleep (LS).

polygraph recordings reveals a combination that does not match any of these stages: low-EEG amplitude (as in PS and W), low-EMG amplitude (as in PS and HS), and low-theta amplitude (as in W) (see Fig. 1). We call this stage "low-amplitude sleep" (LS).[2] In baseline recordings from several studies, we found that LS typically averages ~5% of total time. It typically occurs in short episodes as interruptions of or between other stages. In scoring 30-s epochs from baseline recordings of five rats, we found that only 29.8% of LS epochs were preceded or followed by other LS epochs. Of all LS epochs, 23.6% were preceded by W, 35.5% by HS, and 11.1% by PS; 30.8% were followed by W, 37.5% by HS, and 1.8% by PS.

Since they have not scored it separately, other researchers have apparently combined LS with other stages by unsystematic or unspecified criteria. Although LS comprises a small percentage of total time, systematic treatment of it is of consequence because of the following: (a) Potentially, LS episodes could be combined with any stage. They could be considered W episodes with atypically low EMG, NREM[3] episodes with atypically low EEG, or PS episodes with an atypical paucity of theta activity. Different combinations by different investigators could produce divergent biases of sizable magnitude. (b) Without systematic treatment, the bias could change with experimental expectations, e.g., more scoring of LS as PS following PS deprivation. (c) An erroneous scoring of LS as PS could overscore PS by >50%.

How one scores LS will depend on its relationship to other stages. To elucidate these relationships, we compared LS with W, HS, and PS on (a) spontaneous behavior, (b) response threshold to stimulation, and (c) incidence of "PGO-type waves" (5), i.e., sharp waves in the pons associated with phasic PS brain activity.

STUDY 1: SPONTANEOUS BEHAVIOR

The purpose was to determine whether the rat's behavior during polygraphically scored LS epochs was independently rated as resembling the behavior of W, NREM sleep, or PS.

[2] Our preliminary, noncommital designation of this stage as the "quiet state" (3,4) is no longer necessary, since the present study shows that LS is highly correlated with behavioral sleep.

[3] NREM sleep is here defined conventionally as all sleep that is not PS. Whether NREM sleep in the rat should include periods with low EEG amplitude is the focus of this article.

Electrode implantation

In three male Sprague-Dawley rats, aged 4–9 months, EEG was recorded from two stainless-steel jeweler screws threaded through the skull, the anterior screw 1 mm posterior and 2.5 mm lateral to bregma, and the posterior screw 1 mm anterior and 3.5 mm lateral to lambda. (Lateral placement minimizes the intrusion of theta activity.) For recording theta, two screws were implanted within 1 mm of the skull midline, one screw ~2–3 mm anterior to bregma and the other midway between lambda and bregma. EMG was recorded ipsilaterally from two silver plates (0.5 × 1.5 mm each) cemented to (but insulated from) the temporal skull and resting under one of the temporalis muscles (2). Electrodes were wired to a miniature plug cemented to the rat's head.

Recording

Rats were recorded from a hexagonal glass cage (36 cm high, 32 cm side to side), with food and water available ad lib. A cable attached to the rat's head plug carried the signals to a commutator mounted on a counterbalanced boom, thus allowing the rat free movement about the cage. The signals were carried to a Beckman type R polygraph. EEG was filtered 0.5–15 Hz and recorded at 200 μV/cm. EMG was filtered 5–40 Hz and recorded at 100 μV/cm. Theta was filtered 5–15 Hz and recorded at 200 μV/cm. Records were divided into 30-s epochs by a time mark provided by a PDP 11-10 minicomputer.

Observation of behavior

Mirrors on three sides of the glass recording cage facilitated observation. The cage was placed 15 cm from a one-way window to the observers' room. Two experienced observers, separated by a heavy partition, simultaneously observed the rat through the window. They used separate sets of silent switches independently to indicate, to the nearest half-second, whenever the animal changed behavior and the specific state to which it moved. Observation periods were 1 h long and occurred approximately in the middle of the lights-on portion of a 12:12 light/dark cycle. Observers were denied access to the polygraphic recordings being made simultaneously.

Behavior scoring criteria were as follow: behavioral W—eyes open, either posture erect with head elevated, gross limb movements, or active locomotion; behavioral NREM—prone or curled posture with no apparent resistance to gravity, no gross movements, little or no fine muscle twitches, regular respiration, eyes closed or open (our observations indicate that rats' eyes can be open during sleep); behavioral PS—differentiated from NREM by irregular respiration, numerous fine twitches of the limbs, tail, eyelids, vibrissae, and occasionally the torso, flattening of the ears, absence of piloerection, no maintenance of postural tone.

Electrophysiological records and scoring

Polygraphic recordings during the period of observation were run at paper speeds of 1 mm/s. Five experienced scorers independently classified each epoch of polygraphic record as W, LS, HS, or PS. No polygraph scorer had previous experience in classifying LS. Scorers were given written instructions and scoring examples before scoring began. The instructions (slightly condensed and modified from the original) were as follow: For each of the three signals—EEG, EMG, and theta—there is a low, relatively stable amplitude mode that persists for periods of several seconds to several minutes. This "baseline" mode occurs for EEG and theta during periods when EMG

amplitude is high and variable and for EMG during periods when EEG and theta amplitudes are variable but mostly above baseline. (a) *Score HS* when EEG and theta amplitudes are variable and mostly above baseline for a total of over one-half of the epoch. (b) *Score PS* when EEG and EMG amplitudes are at baseline and simultaneously theta amplitude is above baseline and fairly stable for a total of over one-half of the epoch. (c) *Score W* when EEG and theta amplitudes are at baseline and simultaneously EMG amplitude is above baseline for a total of over one-half of the epoch. (d) *Score LS* when EEG, theta, and EMG are simultaneously at baseline for a total of over one-half of the epoch. If none of the above stages occupies more than one-half of the epoch, score the stage that occupies the greatest part of the epoch.

Results of Study 1

Electrophysiological and behavioral scores were compared for 4 h of recording and observation of each of the three rats. Results were similar for the three rats, so only group data are presented. Observers of behavior agreed with each other on 92.7% of epochs overall, but on only 81.5% of PS epochs. They found an average of 26% W, 67% NREM, and 7% PS. Polygraph scorers found an average of 28% W, 5% LS, 59% HS, and 8% PS. For epochs that a majority (at least three of five) of polygraph scorers agreed were LS, observers scored an average of 10.2% as behavioral W (pooled observations), 87.0% as behavioral NREM, and 2.8% as behavioral PS. Conversely, if we assumed that LS corresponded to NREM behavior and then examined how well majority polygraph scores agreed with one observer or the other, agreement was 94.9% for behavioral W versus polygraphic W, 96.3% for behavioral NREM versus HS and LS, and 84.2% for behavioral PS versus polygraphic PS. Clearly, the behavior of LS was judged much more like that of HS than of W or PS.

STUDY 2: RESPONSE THRESHOLDS

The purpose was to compare response thresholds during polygraphically scored LS with response thresholds during W, HS, and PS.

Methods for Study 2

Four male rats, aged 4–9 months, were implanted for recording as described above. In addition, loops of stranded stainless-steel wire (34 ga., six strands) were implanted subdurally extending down the neck and back between the shoulder blades to serve as shocking electrodes for reinforcement. The rats were recorded from the same hexagonal cage as in Study 1, with the addition of a ¼-in wire mesh grid floor to serve as a second electrode for delivering shock. This grid was cleaned regularly in an acid bath. A 5-cm-high, 0.7-cm-wide plastic barrier was placed across the center of the cage from corner to corner. A beam of light was directed above the barrier's upper edge parallel to it into a photocell to automatically record whenever the rat climbed onto the barrier (the response that was reinforced). A speaker produced low-volume white noise to which a signal could be added as a discriminant stimulus.

EEG, EMG, and theta signals from the polygraph were passed through an A/D converter into a KIM-1 microcomputer programmed to recognize 30-s epochs with "typical" W, LS, HS, and PS electrophysiology. For the first two rats, the program used a D/A converter attached to an audio mixer to generate a 400-Hz tone (discriminant stimulus), which started well below the white noise level and increased exponentially over 6.5 s to maximum amplitude, at which time a 0.1- to 0.2-mA shock was delivered

for 2 s by a Grass stimulator model SD5 plus CCU1A constant-current unit. Rats could terminate the tone shock sequence at any time by mounting the barrier. The program then recorded the response latency and randomly selected both a minimum intertrial interval of 1–32 min and one of the four stages as the next target. The program performed a new response latency test when it recognized an epoch with the target electrophysiology. Rats were overtrained with 200–1,000 trials before testing began. Then each rat was run for a minimum of 20 trials in each stage. The polygraph record was examined each day, and any trial that followed an epoch that did not match the visual scoring criteria, particularly in the 5 s before stimulation, was discarded before the latency data were analyzed. About 25 latencies per day were measured.

During training sessions, rats occasionally failed to respond promptly to widely spaced trials in W, as if they were "inattentive" to the auditory stimulus. This phenomenon has also been reported by Van Twyver and Garrett (6). Since this response failure was not conducive to latency measurement, for the final two rats we changed the discriminant stimulus from the tone to the electric current used to produce shock. By interfacing the constant-current unit to the D/A converter, we were able to produce a current that started below tactile threshold and rose exponentially to the normal shocking current over approximately the same time course the auditory stimulus had followed. This change in procedure eliminated the problem of "inattention."

Results of Study 2

For each rat, PS latency was longer than HS latency and HS latency was longer than W latency ($p < 0.0005$ for each rat), by Tukey's multiple comparison method (TMCM), also known as the "HSD test" (7). A similar result was reported by Van Twyver and Garrett (6). When rats were aroused from PS, they sometimes appeared disoriented or paralyzed, showing signs of arousal, such as eye opening, long before they responded correctly.

For each rat, LS latency was greater than W latency ($p < 0.0005$, TMCM) and less than PS latency ($p < 0.001$, TMCM), but not significantly different from HS latency (at $p < 0.05$, TMCM). Figure 2 shows relative latencies of W, LS, HS, and PS, scaled with W latency = 0 and PS latency = 1. In one rat, LS latency slightly exceeded HS latency, in two rats it was slightly less, and in the fourth rat the latencies were nearly identical. Clearly, the results showed that response latencies during LS resembled those of HS but were higher than those of W and lower than those of PS.

STUDY 3: PGO-TYPE WAVES

The purpose was to compare the incidence of PGO-type (ponto-geniculo-occipital) waves during LS with their incidence during W, HS, and PS.

Methods for Study 3

Five male Sprague-Dawley rats, aged 3–9 months, were implanted as described for Study 1, with the addition of depth electrodes for recording PGO-type waves. These electrodes were constructed from two twisted Teflon-insulated stainless-steel wires (125-μm diameter; Medwire), stripped 0.5 mm at the tip (tips 1 mm apart) and electroplated with platinum for low, stable resistance. Electrode pairs were implanted bilaterally and were stereotaxically oriented to a point 7 mm below the surface of a skull landmark defined as 1.0 mm lateral to the midline and 0.3 mm posterior to the lambda skull suture line, according to the procedure of Marks et al. (8). To confirm electrode

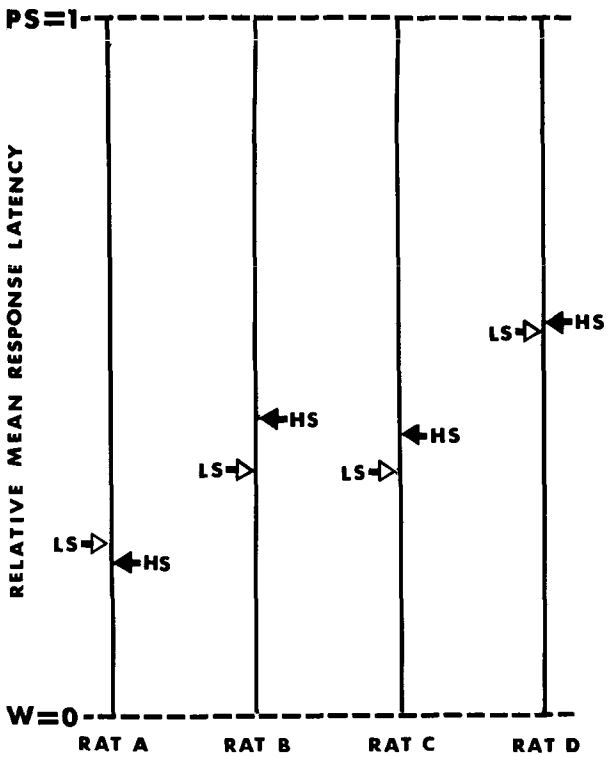

FIG. 2. Relative mean response latency during wakefulness (W; bottom dashed line), paradoxical sleep (PS; top dashed line), high-amplitude sleep (HS; filled arrows), and low-amplitude sleep (LS; open arrows). Relative latencies were scaled with W latency = 0 and PS latency = 1 for each of the four rats.

placement, histology was performed on all rats with 50 cresyl violet–stained frozen sections. Electrode tips were found to be in the ventromedial nucleus (anteroposterior approximately −3.2), the cerebellar peduncles (−3.4 and −2.8), and the locus ceruleus (−2.0 and −2.4).

Rats were placed in a sound-attenuated enclosure and recorded for 3 days each using the techniques described for Study 1. In addition, PGO-type waves were recorded on the polygraph with sensitivities varying across rats and filtering at 5–20 Hz.

Automatic scoring

The various electrophysiological signals were measured as follow: Filtered EEG, EMG, and theta activity were quantified by a resetting integrator-counter system that produced values corresponding to the summed amplitude of each signal for each 30-s epoch. PGO-type waves were quantified by sending the signal through a level detector with its threshold set to give 1–10 spikes per epoch during PS. The waves that passed the detector's criteria were summed in the counter, and the sums for each epoch were sent to a PDP 11-10 minicomputer for storage.

Every day the computer scored the preceding 24 h of recording for W, LS, HS, and PS using the Parametric Animal State Scoring System (PASS) of Bergmann et al. (9,10). Because the PASS method reveals the parametric relations between LS and other stages, and because PASS scoring was used in Study 3, a description of PASS is of value at this point.

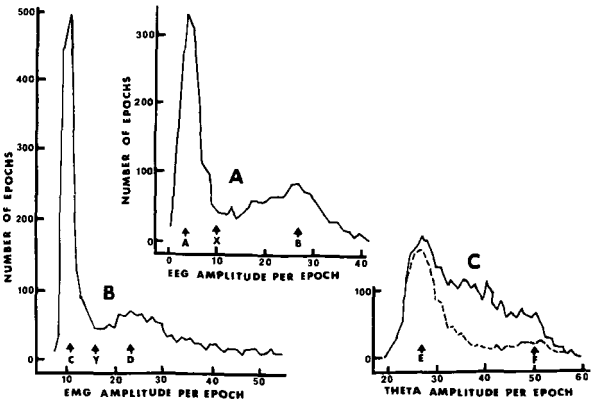

FIG. 3. Frequency distribution of electroencephalographic (EEG; A), electromyographic (EMG; B), and theta (C) amplitudes for 1 day of one rat. Abscissas show amplitude expressed as number of resets of the resetting integrator in a 30-s epoch. Ordinal values show the number of epochs at each amplitude. See the text for description of points A–E, X, Y.

The PASS system uses the total amplitudes of each of three signals (EEG, EMG, and theta) summed across an epoch to score that epoch. For each signal, typical epochs of a stage tend to have similar amplitude values; e.g., PS epochs from a rat have EMG amplitudes clustered around some typical low value, EEG amplitudes also clustered around a low value, and theta amplitudes clustered around some high value characteristic of the particular rat. Such clustering can be seen in frequency distributions (histograms) of large samples of these measures. For example, Fig. 3A is the frequency distribution of 1 day's (2,880 epochs) EEG amplitude per epoch. Clustering, indicated by peaks (modes) in the distribution, is discernible at point A (indicated by arrow), corresponding to the typical EEG amplitude during W, and point B, corresponding to a typical EEG amplitude for HS. Similarly, Fig. 3B shows that the distribution of EMG amplitudes also has clusters corresponding approximately to HS (at point C) and W (at point D). Figure 3C shows two frequency distributions of theta superimposed, one including all 2,880 measures (solid line) and the other a partial frequency distribution including only values from non-HS epochs (dashed line), i.e., those with EEG values below point X in Fig. 3A. In the partial distribution, it is easy to pick out the typical values (centers) of clusters corresponding to W (at point E) and PS (at point F). In general, clusters that are obscured by other clusters will be revealed by using the appropriate partial frequency distribution.

Figure 4 shows a bivariate partial distribution of combined EEG and theta values for sleep epochs only, i.e., epochs whose EMG values are below point Y in the EMG distribution (Fig. 3B). In Fig. 4, each 30 s is represented by a dot showing the EEG and theta amplitudes for that epoch. Three clusters of measures can be seen corresponding respectively to HS, PS, and LS. This clustering would be more prominent if we had eliminated epochs containing transitions between low and high values (e.g., partly LS, partly HS epochs), since such epochs typically fall between clusters. Nevertheless, the parametric basis for separate scoring of LS is clear. LS is unambiguously discriminable from PS on the theta dimension. Although the separation of LS from HS on the EEG dimension is not so discrete, clearly, there is a substantial number of epochs with EMG at the sleep level and EEG amplitudes at the PS level that are not PS.

Sorting epochs into the four stages, i.e., scoring, can be considered geometrically.

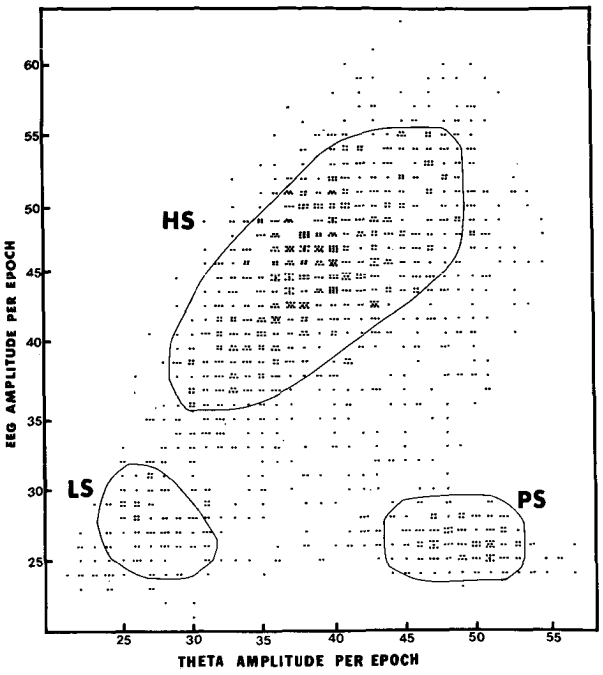

FIG. 4. Bivariate matrix of electroencephalographic (EEG) and theta amplitude values (resetting integrator counts) for all low-electromyographic (sleep) epochs during 1 day in one rat. Each dot shows the EEG and theta amplitude values for one 30-s epoch. Epochs with identical values are plotted immediately adjacent to each other to show density clusters. The cluster outlines shown were arbitrarily drawn for schematic purposes. The quantitative procedures by which cluster centers were identified and individual epochs were assigned to clusters are described in the text. HS, high-amplitude sleep; LS, low-amplitude sleep; PS, paradoxical sleep.

Each stage can be represented by its center point in a three-dimensional (trivariate) distribution. This point will have three coordinates that are the typical EEG, EMG, and theta amplitude values for the stage; e.g., W will have three coordinates corresponding to points A, D, and E in Figure 3A–C, respectively. The centers of the three clusters in Fig. 4 already have EEG and theta coordinates, but need EMG coordinates to completely describe typical HS, PS, and LS.

Computer programs can find the centers of the clusters corresponding to the four stages. Then a program calculates distances to decide which pair of centers (W-LS, HS-PS, etc.) comes closest to the amplitude values of the epoch to be scored. It then chooses as the epoch's stage score the member of the chosen pair that most closely corresponds to those values. This two-stage decision procedure improves the accuracy of scoring epochs that are transitional between two categories by keeping them from being misscored as a third category. For this experiment, a program was also used that matched the incidence of PGO-type waves to the scoring results.

PASS has been validated against the behavioral scoring described in Study 1, where it produced agreement with at least one of two observers 94.0% of the time, compared with 92.5% for the average polygraph scorer. In a comparison to polygraph scorers, PASS agreed 94.1% with stages chosen by a majority of hand scorers, whereas individual polygraph scorers averaged 93.9% agreement. Thus, PASS scoring agrees as well with behavioral stage scoring and average polygraph scoring as individual polygraph scorers (9,10). The good correspondence between PASS scoring and scoring by

visual inspection of polygraph recordings is not surprising, considering that PASS was designed to mimic "visual" scoring by basing decisions on configurations of relative signal amplitudes for a given rat on a given day. Large individual differences among rats preclude scoring by absolute amplitude criteria by either system. For example, as shown in one of our earlier studies (2), integrated NREM EEG amplitude in some rats could be approximately twice as great as in other rats.

Results of Study 3

Table 1 shows the frequency of PGO-type waves per epoch, averaged across four rats, as a fraction of the incidence during PS (determined separately for each day). A fifth rat was excluded from this analysis because of extreme spike artifact during W. These data confirm the report of Farber et al. (5) that PGO-type waves are most frequent during PS. In each rat, daily mean rates of PGO-type waves were significantly lower in LS than in PS ($p < 0.025$, two-tailed paired t), whereas LS rates were always approximately the same as HS rates. PGO-type waves in W ranged from approximately one-seventh to seven times the LS rate across rats because of movement artifact during W in some rats.

Visual inspection of polygraph records convinced us that the difference between the incidence of PGO-type waves in LS and PS was even greater than the automated analysis had indicated. Because LS epochs often contain a small portion of W, HS, or PS electrophysiology, we decided to examine the LS epochs in 2-h samples of each day's record for PGO-type waves in the pure LS segments of these epochs only. Since we expected no movement during LS, we included the data from the rat with extreme movement artifact in W, yielding a total of 30 h (3,600 epochs) examined. In these samples 4.6% of the epochs were LS and 12.6% of these LS epochs contained PGO-type waves. However, only six LS epochs (3.6% of LS epochs) each contained one PGO-type wave during the pure LS segment of the epoch (none showed more), giving these samples an average LS PGO-type wave rate of only 0.005 times the PS rate ($p < 0.001$, two-tailed t). Of these six epochs, five followed HS epochs.

In summary, the incidence of PGO-type waves during LS was much lower than during PS and approximated the incidence during HS. Comparisons with the incidence during W are tentative because of movement artifact.

DISCUSSION

The results show that LS is not a waking state. Relative to wakefulness, LS is a state of profound postural relaxation, motor quiescence, and elevated response threshold. LS is not a low-arousal, motorically quiet waking state; the elevated response threshold decisively indicates behavioral sleep. To reject LS as sleep by denying its

TABLE 1. *Incidence of PGO-type waves in W, HS, and LS expressed as a fraction of the incidence in PS*

	W	HS	LS
Mean (n = 4)	.118	.079	.076
S.D.	.154	.050	.039
*p (df = 3)	<0.10	<0.02	<0.01

* Two-tailed t test for difference from incidence of PGO-type waves in PS.

behavioral attributes would be tantamount to defining sleep by arbitrary electrophysiological criteria alone. Although electrophysiological criteria conventionally define sleep for research purposes, we must periodically remind ourselves that they have acquired their definitional properties from their correlations with behavior. A subsequent reification of electrophysiological definitions in violation of behavioral evidence would amount to stripping "sleep" of all its ordinary meaning, which, since before the advent of electrophysiology, has been primarily behavioral.

The results also indicate that LS is not a variant of PS. Relative to PS, LS shows few muscle twitches, more regular respiration, a lower arousal threshold, and few PGO-type waves—in addition to the reduced theta amplitude used in the definition of the stage.

LS does resemble HS in many ways. The two stages show similar response thresholds, postural relaxation, absence of gross motor activity, paucity of fine muscle twitches, and paucity of PGO-type waves. In fact, of all the parameters evaluated here, only EEG amplitude differentiated LS and HS. It seems unreasonable to preclude LS as a sleep state simply because its EEG amplitude is not elevated. In human studies, Stage 1 and low-amplitude Stage 2 are accepted as sleep stages. Accordingly, it makes sense to consider LS as a stage of NREM sleep.[4]

LS is analogous in several respects to Stage 1 of human sleep. Both have a relatively low-amplitude EEG and occupy a small percentage of total sleep. Both tend to occur for short intervals in the passage from one stage to another or as short interruptions in longer HS or PS episodes.

The acceptance of LS as a stage of NREM sleep in the rat entails new theoretical and practical issues. Should the investigator report LS and HS separately, or is it sufficient to report NREM sleep as a whole? The answer depends upon whether any empirically demonstrated or putative significance is attributed differentially to the NREM stages. For example, we found that in the initial hours following total sleep deprivation, LS was significantly reduced below baseline levels while other stages increased (11). In a pilot study, we restricted HS in a rat by delivering noxious stimuli when EEG amplitude increased. LS progressively increased to where NREM was above baseline levels, despite restricted HS. Nevertheless, progressively more stimulation was needed to block HS, and there was a substantial sleep rebound when stimulation was stopped. Evidently, HS performs some function that LS does not, so it may make sense in some contexts to report LS and HS separately. Under special conditions, the amount of LS may deserve attention in its own right. For example, Nixon and Karnovsky (12) reported long periods of electrophysiology and behavior corresponding to LS after administering piperidine to rats.

The differentiation of LS and PS is perhaps the most important reason for scoring LS. Since LS normally occupies only ~5% of total time and total sleep occupies ~50–60% of total time, an erroneous scoring of LS as W could underestimate total sleep by ~8–10%. This error might not seriously affect experimental results, if it were evenly distributed across experimental conditions. However, since PS normally occupies only ~8% of total time, erroneous scoring of LS as PS could overestimate PS by >50%. A perusal of the literature on rat sleep reveals PS values ranging from ~7 to

[4] Our subdivision of HS into HS1 and HS2 by EEG amplitude alone in other studies (10) is not to be confused with the LS-HS distinction. EEG amplitude is at the same low level during LS, W, and PS; it is higher during HS1 and still higher during HS2.

11%. The lowest values may have been achieved when LS was not scored as PS, and the highest values when it was.

The existence of LS epochs implies that stage scoring in the rat based on recordings from only two sites is in some cases inadequate and in other cases relatively difficult. Recording just EEG and EMG renders LS indistinguishable from PS. Recording just EEG and theta renders LS indistinguishable from W. Recording just theta and EMG makes the differentiation of HS and PS relatively difficult, because the amplitude difference between the stages is usually less on a theta recording than on an EEG recording. Accordingly, three recording sites (EEG, EMG, and theta) are recommended for optimal accuracy.

Finally, a note of caution: Most of what we have reported on LS was based upon ordinary baseline recording conditions. When the temporalis or nuchal EMG level is very low in special conditions, the electrophysiological criteria of LS may be met, although the rat is behaviorally awake. For example, after a rat is startled, the EMG level may first jump and then gradually drop to a very low value as the rat maintains a crouched, oriented position. While depriving rats of sleep on a constantly rotating treadmill, we observed marked increases in recorded LS, although the rats were almost constantly walking. In both cases the EMG of the temporalis and/or the neck muscles could achieve a profound relaxation that was not representative of the overall motor activity of the animal. Under special conditions, each investigator will have to determine how well the electrophysiological measures agree with behavior.

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