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SPONTANEOUS K-COMPLEXES IN BEHAVING RATS

G. MARINI[a*], P. CECCARELLI[a], M. MANCIA[a]

[a] Centro di Ricerca Sperimentale sul Sonno "Giuseppe Moruzzi", Istituto di Fisiologia Umana II, Università degli Studi, Via Mangiagalli 32, 20133 Milano, Italy

INTRODUCTION

Various highly regulated brain oscillations with distinct sites of origin and cellular mechanisms occur in the EEG during NREM sleep, and define it: sleep spindles, delta waves, and the recently revealed slow (< 1Hz) oscillations (21, 1). In addition, electrographic rhythmic patterns known as K-complexes (KCs) are seen in human and cat sleep EEG. The term KC was coined by Loomis *et al.* (13) who, by recording brain rhythms from humans, were the first to report a characteristic "large potential change" when the spindle state (C or 2 stage) of NREM sleep was reached. Rechtshaffen and Kales (18) defined the KC as a transient frontocentral negative/positive signal. The IFSECN glossary (9) stated that KC is "...a high voltage biphasic slow wave frequently associated with a sleep spindle". KCs occur during sleep spontaneously or in response to sudden sensory stimuli.

The functional significance of KCs has still to be clarified. Two proposals have been made. Originally, Loomis *et al.* (13) interpreted these "disturbance patterns" as "forerunners of slow waves", and this interpretation was confirmed by others (23, 2, 3, 7), who proposed that KCs might serve a sleep protection function, preventing unnecessary arousals. Since they can be elicited by auditory stimulation, KCs have been proposed as a physiological correlate of abortive arousal (19, 16, 8). Amzica and Steriade (3), combining simultaneous intracellular and field potential recordings in cats, investigated the underlying neurophysiological mechanisms and presented evidence that KCs may be the EEG expression of the slow (< 1 Hz) oscillation, suggesting a role as carriers of delta waves.

Up to now, no data are available on KCs in rodents-at least to our knowledge. In the course of a study on normal (14) and dysrhythmic (15) oscillations in which long-term multisite video-EEG recordings were made in behaving Sprague-Dawley (SD) rats, we observed well-defined spontaneous KCs during sleep.

The aim of the present study was to definitively identify the KC in the rat. We examined the shape of the KCs, their density, their progression through a full sleep-waking cycle, the spectral components, and the spatial distribution in power spectra, according to the "functional topography" approach (10). This approach suggests that identification of rhythmic EEG components should be based on three main criteria:

* Correspondence to: Dr. G. Marini, Centro di Ricerca Sperimentale sul Sonno "Giuseppe Moruzzi", Istituto di Fisiologia Umana II, Università degli Studi, Via Mangiagalli 32, 20133 Milano, Italy - Tel. +39-02-503-15451 - Fax +39-02-503-15455 - E-mail: gabriella.marini@unimi.it

frequency patterns, spatial distribution over the cortex, and functional reactivity to specific conditions of recording.

METHODS

Animals, surgery, and data recording

Five adult Sprague Dawley (SD) rats (three months old, 225-250 g) were used. All surgical procedures were carried out with full asepsis and in accordance with the UK Animals (Scientific Procedures) Act 1986 and the EEC Council Directive 86/609. All efforts were made to minimize suffering and the number of animals used. Animals were anesthetized with ketamine (10 mg/100 g body weight, i.p.; Ketavet, Gellini, Italy) and additional doses (half the initial dose, i.m.) were given whenever necessary, as judged by the animal's reflexes. A local anesthetic, lidocaine, was applied around surgical incisions. Electrodes were driven into the skull so that the tip just reached the dura. The rats were implanted with multiple electrodes bilaterally along the antero-posterior axes at the locations F1, F2, F7, F8, T3, T4, P3, P4, all against a ground reference (RF) placed in the midline above the cerebellum (see insert in Fig. 1).

Multiple, closely spaced cortical electrodes allowed two-dimensional surface brain mapping of the power spectra distribution. Two silver wires were also inserted into nuchal muscles to record EMG activity. All recordings were monopolar. This approach was selected to enhance expression of synchronous activities since bipolar recordings tend to attenuate the summation of equipotential events. In all the figures, the conventional polarity of the signals (i.e. the negativity upwards) was used.

The freely-moving animals were housed in a Plexiglas cage with food and water *ad libitum*. Video-EEG recordings were obtained during the natural sleep-waking cycle. Recordings were made from each rat from 0900h to 1500h in a natural dark-light cycle, for six consecutive days. The recording procedure has already been described previously (14). Briefly, the electrophysiological signals from the integrated circuit socket were fed through a rotating collector into a 24-channel analog-digital converter (sampling frequency 512Hz sample/channel/s), interfaced with the digitized STARII-Galileo system (EBneuro, Florence, Italy). Data were stored at a resolution of 128Hz.

NREM episodes in the rat last 3-5min on average (5), and when only the deep NREM sleep episodes were investigated, they were found to last less (range 20s-2 min, unpublished results). In order to test the rise and decline of the delta (1-4 Hz) rhythm, only the longest episodes were therefore investigated.

Signal analysis

The NREM sleep stages in the rat were classified according to the following EEG criteria: i) light sleep: gradual increase of voltage and emergence of sleep spindles and delta wave; ii) deep sleep: large amounts of high-amplitude slow wave activity, gradually diminishing until there were no more spindles. The fact that the cortex of rats is ungyrated makes it possible to study the synchrony better.

Spectral analysis was carried out on 30 consecutive artifact-free, 2-s epochs in NREM state in which KCs were present, using the Fast Fourier Transform routine. The power density values were computed from the monopolar leads (F1, F7, T3, P3 in the left hemisphere and F2, F8, P4, T4 in the right hemisphere, all against the ground reference).

To gain more detailed information on the topography of the spontaneous KCs, we studied the spatial distribution of the power spectra. The Star II-Galileo system, using software specifically adapted for rodents (EBNeuro, Florence, Italy), provides the distribution of the power over the frequency range of 0.5-16Hz and for the following frequency bands: 0.5-1 Hz (slow oscillations); 1-4Hz (delta rhythm); 4-8 Hz (theta rhythm); 8-16Hz (corresponding to sleep spindles in the rat: 12-15 Hz, 6). The frequency resolution was 0.5Hz. In order to emphasize the 0.1-4 Hz rhythmic activity, which includes delta waves and slow oscillation, we analyzed this band for seven consecutive 8-s epochs at the frequency resolution of 0.125 Hz.

Quantitative analysis of power spectra from the multiple monopolar EEG leads enabled the computer program to construct brain maps during different cortical rhythms.

These maps show the topographic distribution of spectral power at the frequency bands employed. The use of an automatic color scale in arbitrary units common to all frequency bands helps to visualize the distribution of the absolute power (μV^2). To optimize the contrast in the power spectrum distribution in the 0.1-1Hz frequency band (slow oscillation), this map was also scaled in a color range separately.

RESULTS

Fig. 1 shows that spontaneous well-defined KCs, similar to those recorded from humans and cats, occur in the rat EEG during NREM sleep. KCs consist of two components: a biphasic wave which may be followed by a spindle. The progression of KCs from the early stages of NREM sleep through deep NREM sleep to waking is shown. The onset of sleep coincided with the appearance of a KC (A). During light sleep (B), KCs were few and spindles were maximal. As sleep developed (C), KCs became more numerous, progressively becoming rhythmic, while spindles decreased, and delta waves gradually increased. Just before spontaneous arousal (D), spindles reappeared and KCs could be present, although they were not frequent. KCs were synchronous over all the investigated areas of the cortex though the slow wave and the spindling components were well developed mainly in the frontoparietal leads (F7, F8, T4, see insert in Fig. 1). In posterior leads the amplitude was smaller.

The KC distribution was similar to that observed in humans (3, see Fig. 8). Individual KCs were sometimes more pronounced in one hemisphere or in one lead. Some were occasionally asymmetric.

The shape, the quantitative spectral analysis, and the cerebral maps of KCs are exemplified in Fig. 2 during natural deep NREM sleep (A) and under deep anesthesia (B) in the same chronically-implanted rat. We compared these two states because they are both characterized by a large amount of well-defined KCs. Panel A1 shows the shape of a single KC followed by a spindle during NREM sleep in detail, and panel A2 the power spectrum values of the F7-lead obtained over a 56s period (seven 8-s epochs) in the 0.1-4Hz band. There is a major peak at 1.75 Hz and several compound peaks between 1 and 4 Hz. An additional component at 0.75 Hz represents the slow (< 1 Hz) oscillation. Its frequency corresponds to the 0.70 Hz oscillation reported in the cat (3), and the 0.79 Hz oscillation in humans (1). The brain mapping based on quantitative spectral analysis, and scaled in a color range common to all frequency bands, is shown in panel A3. In the 1-4 Hz frequency band, the power spectrum values were highest in the F7, F8, and T4 leads. Since the power spectrum distribution in the 0.1-1 Hz frequency band was not clearly detected, to optimize the contrast, this map was scaled in a color range separately. The spectral power distribution was similar to the 1-4Hz band (not illustrated). There is slight variability in the power spectrum distribution of KC intra- and interanimals.

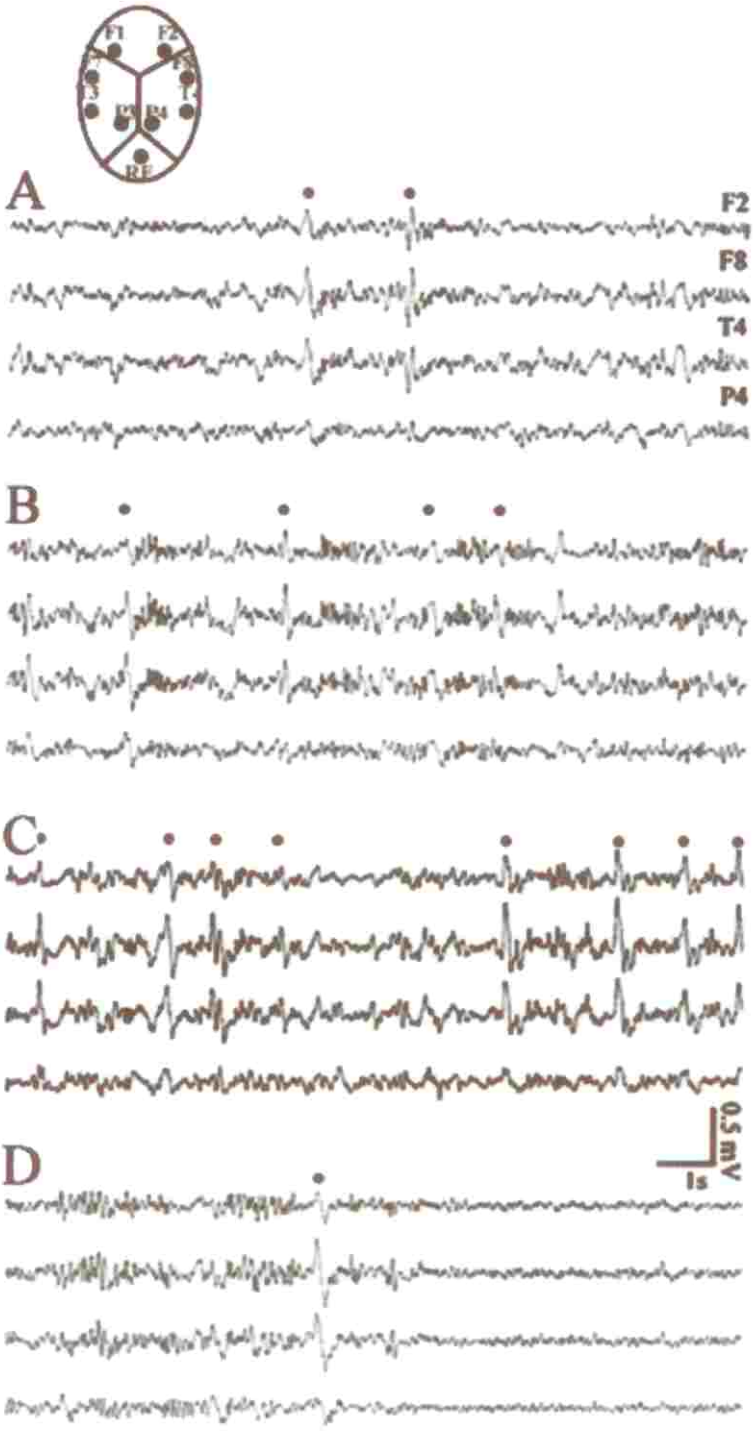

Fig. 1. - *Rhythmic K-complexes (KCs) in rat EEG during a sleep-waking cycle, showing their dynamic progression.*

Monopolar recordings from F2, F8, T4, P4 (right hemisphere) in a representative behaving rat. The placement of the electrodes on the scalp is illustrated in the inserted figurine. Dots mark the most obvious KCs in order to suggest the pattern of their rhythm. (A) One KC marks the onset of the EEG synchronization (onset of NREM sleep). (B) The gradually increased synchronization (light NREM sleep) is evident. Note the progressive increase of the KCs. The amplitude of the accompanying spindles is maximal. (C) Maximal EEG synchronization (deep sleep) is shown: KCs are more numerous, and spindle amplitude decreases until there are no more. (D) Spindles reappear and a single KC occurs just before the awakening. Note that KCs were synchronous over all the investigated areas of the cortex even though the slow wave and the spindling components were well developed mainly in the frontoparietal leads (F8, T4, see head figurine). In posterior leads they were of smaller amplitude.

Panel B1 shows one single KC recorded from the same rat during deep ketamine anesthesia. The electrographic pattern resembled that during NREM sleep (A1). The incidence as well as the amplitude (0.30/s and 212.1 μV for anesthesia, and 0.32/s and 209.5 μV for NREM sleep), refer to values calculated over ten 56-s periods from the F7- lead. However, the frequency of both biphasic wave (2.7 Hz) and associat-

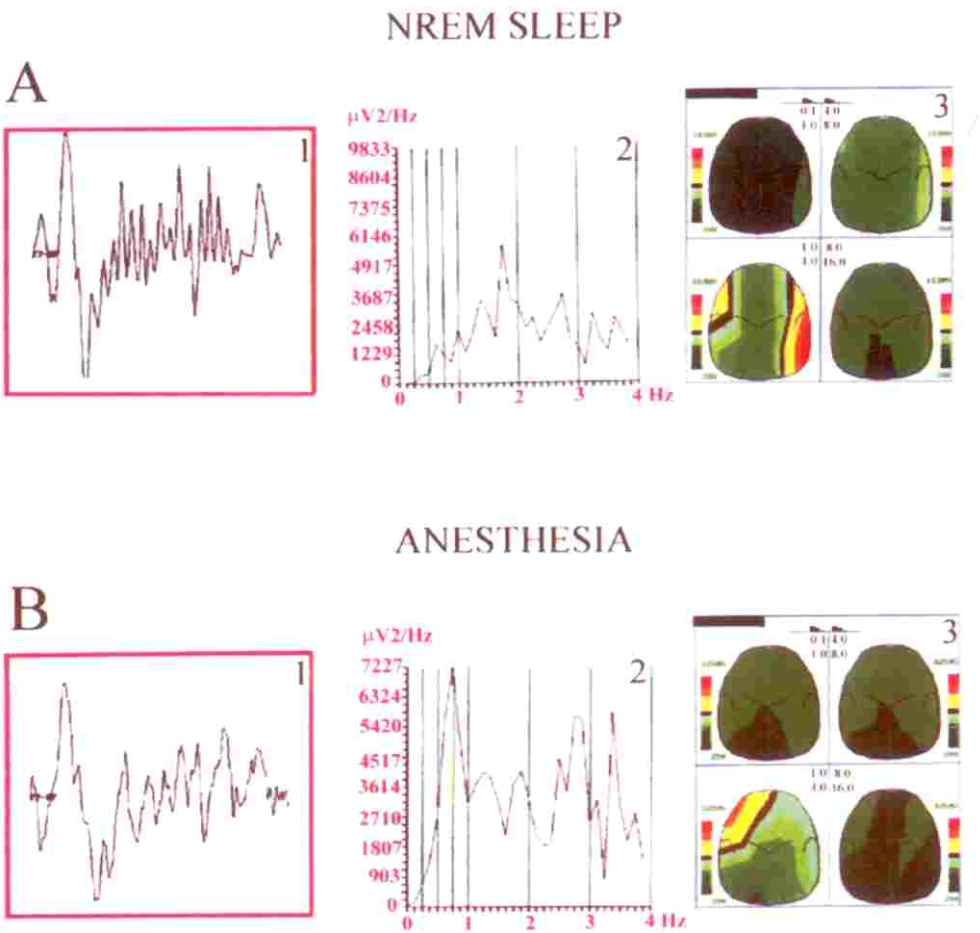

Fig. 2. - *K-complexes (KCs) during natural NREM sleep (A) and under anesthesia (B) in the same representative rat.*

A1: shape (biphasic wave followed by a spindle) of a representative KC during natural NREM deep sleep; B1: under deep anesthesia. Under anesthesia the frequency of the biphasic wave and the associated spindle are slower than during natural sleep. A2 and B2 are the NREM sleep and anesthesia power calculated from F7 lead up to 4Hz over seven epochs of 8-s epochs containing KCs. During natural sleep, the major peak is at 1.75 Hz, surrounded by other lower peaks and a minor component at 0.75 Hz corresponding to the slow (< 1 Hz) oscillation. Under anesthesia, the major peak shifted at 0.75 Hz. A3 and B3 illustrate the topographic distribution of the power in the same conditions. During NREM sleep, the values of the power spectrum are highest in the 1-4 Hz frequency band at the F7, F8, T4 leads. Under anesthesia, more frontal regions are involved, with asymmetric power distribution.

ed spindle (10 Hz) is slower as compared to NREM sleep values (3.40Hz and 12.07 Hz respectively). In B2, the striking peak at 0.75 Hz can be appreciated better and there was a shift towards slower frequencies. In B3, the brain mapping showed an involvement of more frontal regions, and there was some asymmetry.

The typical rapid rising trend with fluctuations of the delta activity during one entire episode of NREM sleep (22) was confirmed in episodes of deep NREM sleep.

Evoked KCs were never observed, but this would in fact be rather an exception in a sound-free environment where sleep is investigated. In addition, no overt motor correlates of KCs were ever seen.

DISCUSSION

The K-complex (KC) has been recorded in the human and cat sleep EEG (13, 2). The main finding of the present study is that spontaneous well-defined rhythmic KCs also occur during sleep in freely moving SD rats. This is the first report-at least to our knowledge- of KCs in the rat EEG, although Pinault *et al.* (17, see Fig. 1) briefly mentioned the occurrence of a KC in Wistar rats. It is worth recalling that our EEG recordings were all monopolar. In previous studies of the sleep EEG, the use of bipolar recordings, which tend to attenuate and hide synchronous equipotential events, may have made the KCs difficult to detect.

The criteria for KC recognition were based on the IFSECN (9) definition as an EEG pattern consisting of "a high voltage biphasic slow wave frequently associated with a sleep spindle". Our results showed that the rat KC shares similar features with the human and cat KC as concerns the shape, the spectral content, and patterns of appearance across conditions. In addition, the study indicated that the slow (< 1 Hz) cortical oscillation observed in cats and human subjects (21, 1) was also present synchronously in all investigated brain areas of rats, with similar temporal characteristics.

So far the topography of spontaneous KCs have received limited attention. The present polygraphic multisite recordings provide some detailed information even though no recordings from midline regions were taken in our experimental conditions. The KCs were synchronous and present in all leads. However, their amplitude reached a maximum in the frontoparietal (F7, F8, T4) and diminished in more anterior (F1, F2) and posterior (P3, P4) leads. The brain maps, based on the quantitative spectral analysis, also showed that the highest values of the power spectrum of KC prevailed in the frontoparietal cortex (see 20). These results are in accordance with and extend those of Loomis *et al.* (13), suggesting that the human KC was most prominent on top, less in front, and least on the back of heads and with Amzica and Steriade (2), who showed a clear diminution in amplitude from the vertex (C3 and C4) towards the occiput (O1 and O2). The highest power values in the parietal region observed in the mapping studies may be a consequence of anatomical features such as the large ascending projections from anterior thalamic nuclei.

Another finding worth mentioning is that, comparing the EEG patterns during natural sleep and under anesthesia, KCs had common features as regards the shape

and the spectral content. However, under anesthesia, the topographic distribution of the power was more frontally located and asymmetric.

The presence of KC and slow (< 1 Hz) oscillation across mammalian species with different cortical extension confirms that these rhythmic activities have great potential functional significance. The question is still not clear, however, what their function is.

As mentioned in the introduction, the original interpretation by Loomis *et al.* (13) of KC as reflecting a sleep protection function has been confirmed in several studies (23, 2, 3, 7). Recent evidence indicates that KCs reflect a brain state in which arousals are less likely to occur (see 4). In accordance with this, we found a maximum amount of KCs during deep sleep and anesthesia, when arousability is minimal. Others, based on KC elicited by auditory stimulation, considered these complexes as a physiological correlate of excitatory events (19, 16, 8).

From our continuous long-term video-EEG monitoring in the behaving rats we noted that: 1) KCs are true "sleep figures", since they consistently precede the first spindle and their density increases as NREM sleep gets deeper, as occurs with delta waves (12); 2) the incidence of KC gradually increased as ketamine-anesthesia deepened-condition in which delta waves are increased (11), 3) the relation between KC and activation is not always evident and no motor correlates were ever associated with them; 4) further support comes from the spectral analysis data showing that the KC always contributes to the power spectrum of the delta band.

These data argue for a role of KC as precursor of slow waves, in line with a clinical study that found that patients with a sleep disorder have significantly less well-defined KCs than normal (23). These Authors suggested that the patients might be less able to produce KCs because their brains are less able to produce slow wave. However, the fact that we found KCs-even though sporadic- before arousal fits in with the possibility of some association between the KC and arousal (19), but it makes it difficult to consider KC as a correlate of a sleep-protecting mechanism. The question of the KCs functional role is still to be conclusively solved.

SUMMARY

The K-complex (KC) is an electrographic rhythmic pattern present in human and cat sleep EEG. In long-term multisite videoEEG recordings in behaving Sprague-Dawley (SD) rats, well-defined spontaneous KCs were observed during sleep.

Sprague-Dawley rats were implanted with multiple electrodes bilaterally along the antero-posterior axes at the locations F1, F2, F7, F8, T3, T4, P3, P4, all against a ground reference placed in the midline above the cerebellum. Multiple, closely spaced cortical electrodes allowed two-dimensional surface brain mapping of the power spectra distribution.

Two silver wires were also inserted into nuchal muscles to record EMG activity. Each rat was monopolarly recorded from 0900h to 1500h in a natural dark-light cycle for six consecutive days. In view of the lack of available data on KC in

rodents, we examined the patterns of appearance in various conditions, the progression through a full sleep-waking cycle, the shape, density, spectral components, and spatial distribution in power spectra. The rat KC appears to share similar features with the human and cat KC.

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