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# FIRING PROPERTIES OF CAT BASAL FOREBRAIN NEURONES DURING SLEEP-WAKEFULNESS CYCLE

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The electrical (Sterman and Clemente 1962a, b; Obál et al. 1980; Benedek et al. 1981) and chemical (Hernandez-Peón 1962) stimulations of different regions in the basal forebrain area (BFA) induce synchronization and natural sleep in the behaving cat. The destruction of this area is followed by insomnia or sleep disturbances (McGinty and Sterman 1968; Lucas and Sterman 1975). These results strongly suggest that the BFA is involved in sleep regulating mechanisms. However, there are no data about the modifications of neuronal activity in the BFA during the sleep-wakefulness cycle. The only exception is a paper by Parmeggiani and Franzini (1971), but this report deals mainly with anterior hypothalamic neurones. Therefore, the aim of the present experiments was to study the neuronal firing patterns in the preoptic area (POA) and olfactory tubercle (OT), regions which were found to be effective in eliciting cortical synchronization and sleep (Sterman and Clemente 1962a, b; Obál et al. 1980).

## Methods

The experiments were performed on 8 freely moving cats of both sexes. Under Nembutal anaesthesia the cats were implanted with cortical, ocular and muscular electrodes for sleep monitoring. A small mechanical microdrive containing 4 teflon-coated stainless steel microwires (diameter 75 $\mu$m) was cemented on the skull above the POA or OT.

The animals were allowed to recover for at least 1 week after the operation. The experiments were performed in daily sessions between 9 and 12 a.m. The EEG, EMG and EOG were recorded by a Beckman polygraph. The neuronal activity from all of the 4 electrodes was stored synchronously on magnetic tape. Records from each well established phase of the sleep-wakefulness cycle, continuous if possible, were obtained from each explored site. After successful recording, the electrodes were advanced a few hundred microns. This new point was examined in the experiment of the next day. The last recording site was labelled in each electrode track: a small amount of iron was deposited by passing a 50 $\mu$A current for 20 sec through the electrode tips. After the completion of the experiments the animals were anaesthetized with Nembutal and perfused through the carotid arteries with 10% formalin solution containing 2% potassium ferrocyanide to give the Prussian blue reaction with the iron deposited previously. The brain was removed and the required part was embedded in paraffin. The locations of the recording sites were verified according to the stereotaxic atlas of Jasper and Ajmone Marsan (1955) in 10 $\mu$m thick sections stained with cresyl violet.

In the course of the quantitative evaluation of the data, quiet wakefulness without movements (W), slow wave sleep (SWS) and paradoxical sleep (PS) were distinguished on the polygraphic record according to the usual criteria. The simultaneous registration of a marker signal every 30 sec on the polygraph and on the magnetic tape made it possible to relate the polygraphic and spike events.

One electrode picked up the activity of several neurones at the same time. To analyse independently the activity of these neurones, a special interface was developed in our laboratory, which enabled the measurement of the 3 least correlated

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parameters of each spike (Vibert and Costa 1979) exceeding an adjustable threshold. The measured parameters are: the amplitude of the first (A1) and second (A2) peaks of the mostly biphasic discharge, and the time (DT) elapsing between them. The 3 parameters determine a 3-dimensional vector, the orientation of which characterizes the shape of the spike. Since the endpoints of the vectors belonging to similarly shaped spikes tend to form clusters in the 3-dimensional space, their projections are also clustered on the 3 planes determined by the parameter pairs (Fig. 1). These clusters can be delimited with ellipses which are the projections of the ellipsoid enclosing the endpoints of the clustered vectors in the 3-dimensional space.

The evaluation of the spike data was accomplished as follows. First, the raw data were fed into the 4 parallel channels of the interface and the thresholds of the channels were set independently above the noise level, then the computer measured and stored on digital tape continuously 5 data about each spike: the serial number of the input channel, the moment of spike occurrence measured from the beginning of data conversion, and the 3 parameters of the spike shape mentioned above. After all the data obtained from one recording point had been stored, the computer constructed and displayed from selected parts of the data the 3 projections of the endpoints of the vectors representing the spikes in the 3-dimensional parameter space. The structures of clusters from different periods (W, SWS, PS, the beginning and the end of the record) were compared with each other, and only those records showing only slight modifications were further analysed. In some cases the spike amplitude was slightly increased in W, but this did not influence the separation. Interacting with the computer, the clusters were delimited on the screen with ellipses, the parameters of which were used for the separation of discharges into groups during the evaluation. It was assumed that the discharges enclosed in one ellipsoid were generated by the same neurone. To test this assumption parts of the raw data were fed again into the interface and through an analog delay circuit (Bak and Schmidt 1977) to a storage oscilloscope, which got a trigger signal from the computer whenever the spike fitted into the group

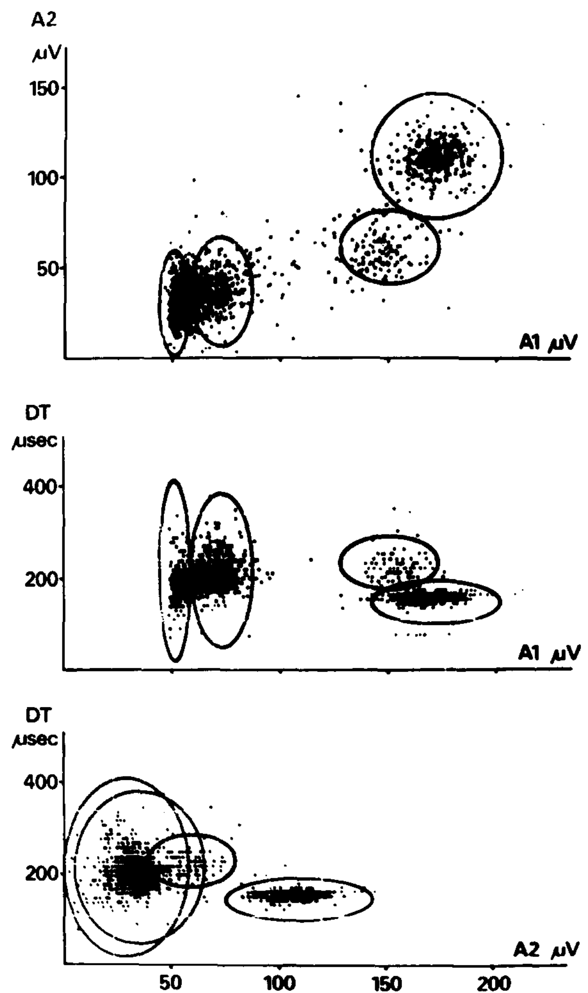


Fig. 1. The endpoints of the vectors belonging to similarly shaped discharges tend to form clusters in the space spanned by the 3 measured parameters of the spike shape: the amplitudes of the first (A1) and second (A2) peaks, and the time (DT) elapsing between them. The projections of these clusters onto the 3 planes determined by the parameter pairs are delimited with ellipses on the oscilloscope screen using an interacting computer program. The equation of ellipses confirmed later to encircle the discharges of a single unit are used as separation criteria.

selected previously. If the overlap of the consecutive spikes plotted on each other was not appropriate and the modification of the parameters yielded unsatisfactory result, the group was neglected.

After completion of the separation procedure, 3

periods of 50 sec length were chosen from each well established phase of the sleep-wakefulness cycle (W, SWS, PS), and firing frequencies, interval histograms and autocorrelograms were computed for the individual neurones. The firing frequencies belonging to the same cell and to the same phase were pooled, and their averages were compared with each other. During comparison only those differences exceeding the arbitrary limit of 10% were considered as changes. The interval histograms and the autocorrelograms were plotted by the computer on an X-Y recorder and visually evaluated. These histograms provided a second possibility to test the appropriateness of spike grouping. It seems that every discharge of a neurone should be followed by a few milliseconds of silent period; that is, the first few bins of the histogram should be zero. If this is not the case, it may be supposed that discharges of several neurones are pooled in the same group.

To yield information about the timing of the firing frequency changes cumulative discharge curves were constructed for the individual neurones around the SWS → PS transition in every case when a continuous record was at our disposal. As it seemed difficult to determine the exact moment of W → SWS transition it was not investigated. The onset of PS was established on the polygraphic record as the moment of EEG desynchronization. This moment was transferred to the cumulative discharge curve with the aid of the marker signals. The cumulative discharge curves were visually evaluated: the point where the curve began to decline from linear was defined as the beginning of the frequency change (Fig. 4).

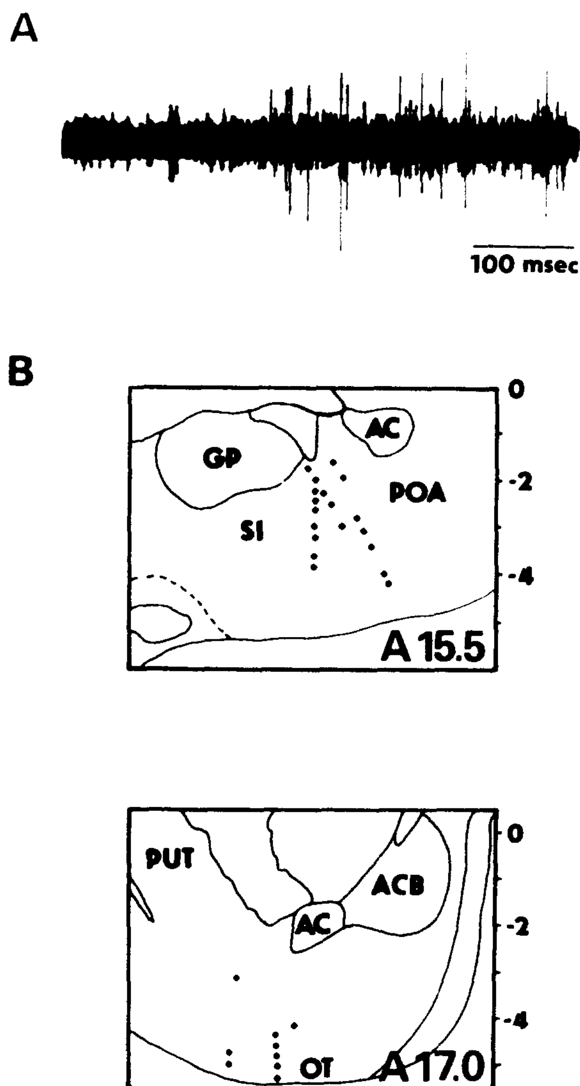


Fig. 2. Example of the original record (A) and the distribution of recording sites in the two examined areas, POA and OT (B).

## Results

The activities of 62 cells from 19 points of the POA (at A 15.5) and those of 46 units from 9 sites of the OT (at A 17.0) were analysed quantitatively. The anatomical localizations of the recording sites and an example of the recorded activity are shown on Fig. 2.

All of the examined cells were active during both wakefulness and sleep; in most cases there were only moderate changes in the activity when passing from one stage into the other. The average firing frequency of POA units in W was 19.2 c/sec, the median 15.3 c/sec (Fig. 3). Most of the neurones either did not change or somewhat lowered their activity when the cat entered SWS (Table I). This fact is reflected in the slight decrease of average frequency and median values. However, the distributions of firing frequencies were very similar in the two stages, the peak value (mode) being in the 12–16 c/sec range. In con-

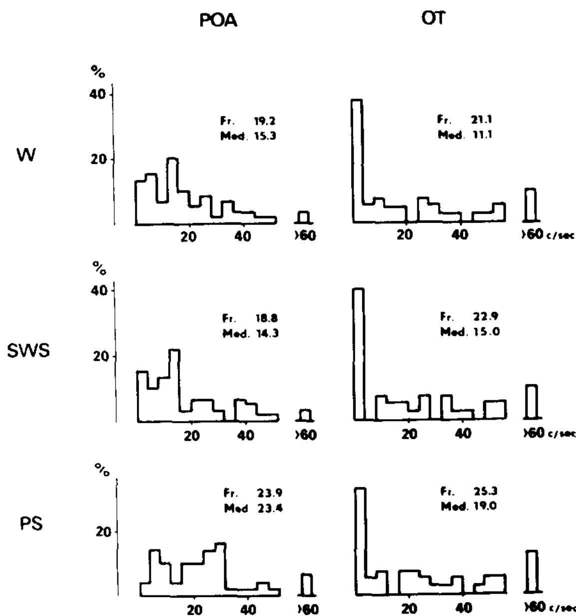


Fig. 3. Distribution of firing frequencies displayed by the POA and OT units in the 3 phases of the sleep-wakefulness cycle. The values show the percentages of total recorded cells. Fr., average firing frequency; Med., median.

trast, paradoxical sleep brought about a considerable increment in average firing and median values and a clear-cut change in the shape of the distribution: it became bimodal, with one peak in the 4–8 c/sec, the other in the 28–32 c/sec, ranges. This bifurcation was caused by some neurones displaying a roughly 2-fold increase in firing frequency from about 12 c/sec in SWS to about 24 c/sec in PS. This change was the largest in the whole material at any phase transition, the other differences reaching in most cases only 30–40%. Nevertheless, almost all of the POA cells (91.2%) were activated in paradoxical sleep compared to slow wave sleep, and this activity in most cases (88.2%) was also greater than in quiet wakefulness.

The firing characteristics of OT neurones were quite different from those of POA units. In W the average firing frequencies in the two areas were similar (Fig. 3), but the median of OT cells was much lower because of the high proportion of units firing in the 0–4 c/sec range. The approximately equal average firing in POA and OT is explained by the existence of a larger population in the latter discharging above 60 c/sec. Compared to W, the activity level was higher in SWS in almost half of the neurones (43.6%) (Table I). This caused an increment in the average firing frequency and a more pronounced rise in the value of the median. During paradoxical sleep, both measures were further elevated, in spite of the fact that in OT only half of the cells (51.3%) were activated in PS compared to SWS. Similarly, in contrast to the case in POA, a much lower proportion of the neurones (50.4%) displayed higher activity during paradoxical sleep than in quiet wakefulness.

The timing of the frequency changes during the SWS → PS transition was examined on the cumulative discharge curves (Fig. 4). These curves were constructed only for those neurones having different firing rates in SWS and PS. In POA the majority of the examined cells (95.7%) had a higher activity in PS, and the frequency change in most cases (78.3%) took place after the appearance of EEG desynchronization. The increased activity preceded this moment in only 17.4% of the units. The remaining few cells (4.3%) decreased their activity during PS. The decrease was observed before the first sign of paradoxical sleep. None of the neurones decreased its firing after PS was established.

In OT 91% of neurones for which a cumulative

TABLE I

The percentages of POA and OT cells displaying higher, lower or equal firing frequencies comparing one state of the sleep-wakefulness cycle with the others.

| | SWS vs. W | | PS vs. SWS | | PS vs. W | |
|---|---|---|---|---|---|---|
| | POA (%) | OT (%) | POA (%) | OT (%) | POA (%) | OT (%) |
| Higher | 17.4 | 43.6 | 91.2 | 51.3 | 88.2 | 56.4 |
| Lower | 34.8 | 28.2 | 2.9 | 20.5 | 2.9 | 20.3 |
| Equal | 47.8 | 28.2 | 5.9 | 28.2 | 8.8 | 33.3 |

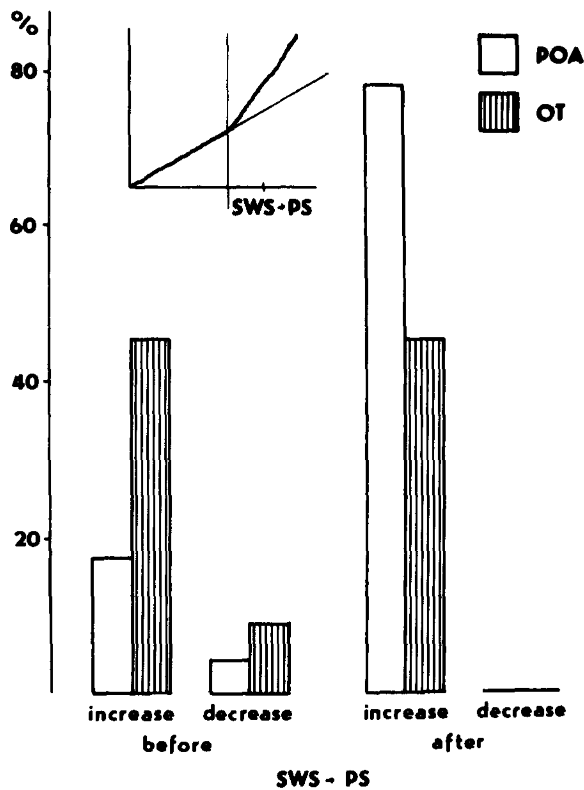


Fig. 4. Types of frequency change during the SWS → PS transition. Insert shows determination of the beginning of the frequency change on a cumulative discharge curve.

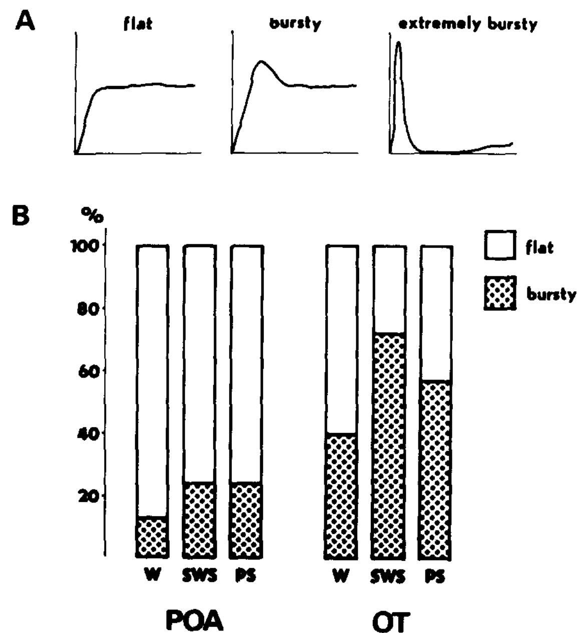


Fig. 5. Autocorrelogram types (A) and their proportion in POA and in OT in the 3 stages of the sleep-wakefulness cycle (B).

discharge curve could be constructed increased their firing rate during the SWS → PS transition. Half of them (45.5%) were activated before the beginning of PS, the other half (45.5%) after that moment. The decrements (9.1%) always preceded the PS, just as in POA units. Most of the changes appeared within the range of 5–15 sec before or after the moment of phase transition.

In most cases the spikes were evenly spaced or, especially in SWS, showed a slight tendency to form clusters. This tendency was not as strong as observed, for example, in thalamic neurones during SWS, but could be clearly seen in the shape of autocorrelograms. Three types of correlogram were observed (Fig. 5A): (1) flat — the firing probability rose to a constant level after a short refractory period; (2) bursty — similar to the preceding one, but the firing probability reached a peak before it declined to a constant level; (3) extremely bursty — the peak was very high and narrow and was followed by a period of diminished firing probability.

This latter type occurred only on a few occasions, exclusively in SWS; therefore the two bursty groups are summed in Fig. 5B. The spikes of the POA neurones were mostly evenly spaced: this is reflected in the predominance of flat type autocorrelograms. In contrast, most of the OT neurones fired in clusters during slow wave sleep, the proportion of bursting neurones being high even in wakefulness and in paradoxical sleep.

## Discussion

On the basis of the stimulation and destruction experiments it could be supposed that there are neuronal populations in both POA (Sterman and Clemente 1962a, b; McGinty and Sterman 1968; Lucas and Sterman 1975) and OT (Obál et al. 1980; Benedek et al. 1981) which are involved in the priming mechanisms of synchronization and

sleep. In the present study the unit activity was recorded from both structures during the sleep-wakefulness cycle. We did not find, however, clear-cut correlations between the alteration of neuronal activity and the development of sleep stages. Most of the POA neurones (82.6%) either did not change or decreased their firing rates at W → SWS transition. In contrast, 43.6% of OT cells were activated in SWS, but their frequency change was very small. It seems quite improbable that such a moderate activation could play an important role in synchronization. However, the existence of a synchronizing and hypnogenic neuronal population cannot be excluded. It may be located in another, not investigated, part of BFA or may consist of small cells, missed due to sampling bias of the microelectrode technique. A further possibility is that the stimulation and destruction experiments influenced fibres crossing this area.

It could be supposed that afferent influences from the brain-stem play a certain role in modification of the activity of BFA neurones. On this basis, for example, the frequency changes in the discharge of OT cells could be explained as follows. The neurones of the nucleus raphe dorsalis are known to project to BFA (Bobillier et al. 1976); 5-HT has a depressing effect on neuronal firing in the basal forebrain area (Bloom et al. 1973). Therefore, the monotonous frequency increase in the course of the W → SWS → PS sequence in a great number of OT cells may be considered as a gradual disinhibition from the influence of 5-HT, since the raphe neurones decrease their firing rate during sleep (Trulson and Jacobs, 1979).

The differences in the timing and in the nature of firing frequency changes during sleep between the two investigated areas clearly suggest that they may have different functional organizations and roles. This is further supported by the predominance of the stochastic firing pattern of POA units and by the bursting pattern in the activity of OT cells. These differences make it difficult to find a uniform explanation for the synchronization and sleep elicited by stimulation of the two areas and give further support to our suggestion that the activation of cells investigated may not be inolved in the induction of synchronization and sleep. Therefore, the problem of the functional relationship between the BFA and the sleep mechanisms remains to be solved.

**Summary**

Neuronal activity was studied in the basal forebrain area (BFA) of freely moving cats during wakefulness (W), slow wave sleep (SWS) and paradoxical sleep (PS). Two classically synchronizing and hypnogenic regions, the preoptic area (POA) and the olfactory tubercle (OT) were explored by microelectrodes. Compared to W, the discharge rate in most of the POA cells was not modified or was slightly reduced by SWS, but it was increased by PS. Half of the OT cells increased slightly their firing frequency during falling asleep. A great proportion of OT neurones showed facilitation of activity during PS also, which in half of the cells started already in the last seconds of SWS. The results are discussed from the point of view of the synchronizing and hypnogenic influence attributed to POA and OT.

**Résumé**

*Propriétés des décharges neuroniques dans l'aire basale du cerveau chez le chat au cours du cycle de veille-sommeil*

On a étudié l'activité neuronique dans l'aire basale du cerveau pendant l'éveil, le sommeil lent (SL) et le sommeil paradoxale (SP) chez le chat libre. Deux régions synchronisatrices et hypnogènes, l'aire préoptique (AP) et le tubercule olfactif (TO), ont été explorées par microélectrodes. Par comparaison avec l'éveil, la fréquence de décharge de la majorité des cellules dans l'AP est réduite, dans une mesure modérée, ou n'est pas modifiée pendant le SL, mais est augmentée pendant le SP. La moitié des neurones du TO augmente leur fréquence de décharge pendant l'endormissement. Une grande part des cellules du TO montre une facilitation de leur activité, s'installant déjà pendant les dernières secondes du SL. Les résultats

sont discutés en fonction de l'influence synchronisatrice et hypnogène, attribuée à l'AP et au TO.

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