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Neuronal firing in the pallidal region: firing patterns during sleep-wakefulness cycle in cats

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Summary Neuronal activity was investigated by extracellular microelectrodes in the pallidal region of freely moving cats during wakefulness (W), slow-wave sleep (SWS) and paradoxical sleep (PS). The firing of 150 units from 35 points was examined. On the basis of the modifications of firing rates and patterns during the sleep-wakefulness cycle, 5 groups of neurons were distinguished. Two of these groups were characterized by strong increase of firing rate in W and PS and in one of them this increase preceded the cortical activation at the SWS-PS transition by an average of 26 sec. The role played by the basal forebrain area in the regulation of the sleep-wakefulness cycle is discussed.

Key words: Pallidal region; Unit activity; Desynchronization; Sleep-wakefulness cycle; Cat

A number of findings indicate that the basal forebrain area (BFA) is involved in the control of sleep and wakefulness. Stimulation of the preoptic area (POA) (Sterman and Clemente 1963; Hernandez-Peon 1965) and olfactory tubercle (TO) (Obal et al. 1980; Benedek et al. 1981) induces cortical synchronization and sleep. Bilateral electrolytic lesions of the BFA result in insomnia and sleep disturbances (McGinty and Sterman 1968; Lucas and Sterman 1975). On the other hand, the recently described basal forebrain cholinergic system (BFCS) (Kimura et al. 1981), which is the only major source of cortical ACh (Johnston et al. 1981), seems to be involved in cortical activation: the acetylcholine release from the cortex increases manifold during desynchronization (Szerb 1967; Jasper and Tessier 1971); the administration of atropinic drugs produces large slow waves even during wakefulness (see Vanderwolf and Robinson 1981); and the injection of the neurotoxin, kainic acid into the BFA abolishes an atropine-sensitive component of desynchronization in rats (Stewart et al. 1984).

There has been only limited investigation of the neuronal events occurring in the BFA during sleep and wakefulness (Parmeggiani and Franzini 1971; Mallick et al. 1983; Kaitin 1984; Szymusiak and McGinty 1986). For that reason, in a previous study we investigated the neuronal firing in the two classical hypnogenic structures, POA and TO during the sleep-wakefulness cycle (Detari et al. 1984). Continuing this mapping, we examined the pallidal region in the present experiments, as this area was shown to contain large neurons of the BFCS (Kimura et al. 1981). The main target was the border of the putamen and the globus pallidus where the cholinergic cells were found in a more or less vertical column. This fact increased the probability of encountering them during the vertical electrode descent. Part of the results has been briefly reported elsewhere (Detari et al. 1985).

Methods

The experiments were performed on 8 unrestrained cats of both sexes aged between 2 and 4

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years weighing 2.5–4.0 kg. All the experimental procedures were described earlier (Detari et al. 1984). Therefore, only a brief outline of this description will be presented below.

Surgical and experimental procedures

Under Nembutal anesthesia a movable microwire bundle (4 × 75 μm, teflon-insulated stainless steel) was implanted for unit recordings together with the usual macroelectrodes for sleep recordings: screw electrodes in the primary somatosensory (SI) and occipital (Occ) cortices to record the electrocorticogram (EEG), among the eye muscles to record the electrooculogram (EOG) and pin electrodes in the neck muscles to monitor muscle tone (EMG). The animals were housed in the experimental chamber during the 1 week recovery period and during the experiments. Throughout the daily recording sessions (between 9.00 and 12.00 a.m.) the EEG, EMG and EOG signals were recorded continuously on a polygraph. The spontaneous neuronal activity from the 4 microelectrodes and the cortical EEG (SI-OCC) signal were stored on analog tape in the course of one or two sleep-wakefulness cycles. The electrodes were lowered by 250 μm at the end of each recording session. The new point was examined on a separate day. Once in the target area, at least one of the electrodes picked up good neuronal activity in most cases. Therefore, it was not necessary to search for units before the recording sessions. At the conclusion of the experiments on a given cat, the last recording site in each electrode track was marked by a small lesion and histologically verified.

Data processing

Generally, one electrode recorded the activity of several neurons simultaneously. The unit discharges stored on the magnetic tape were separated by wave shape and duration using a special interface and a minicomputer. The spike shapes were characterized by measuring 3 parameters: the amplitude of the first, usually negative (A1) and the following, usually positive (A2) peaks and the time (DT) elapsing between them. The whole signal was inverted during the processing, if necessary. From selected parts of the recording the computer constructed 3 plots (A1-A2, A1-DT, A2-DT). The points representing similarly shaped spikes generated supposedly by the same neuron were clustered on these plots and could be delimited with ellipses. The equations of these ellipses were used as criteria for separation of units during the subsequent data processing. To check the stability of spike shapes, plots were constructed and compared from different epochs of the original recording. The variability of discharges was examined by superimposing several spikes on the screen of a storage oscilloscope. The oscilloscope displayed the original recording from the tape after passage through an analog delay circuit (Bak and Schmidt 1977) and triggered by the computer whenever a spike fitted into the ellipse selected previously (Fig. 1 and Detari et al. 1984).

The parameters and patterns of firing were determined for each unit in 3 states of the sleep-wakefulness cycle: in quiet wakefulness without movements but with desynchronized cortical EEG (W), slow-wave sleep (SWS) and paradoxical sleep (PS). For each state 3 samples of 50 sec length were selected randomly and interval histograms as well as autocorrelograms were constructed and plotted on an X-Y recorder in 4 resolutions (0.5, 2, 8, 32 msec/bin) for the individual neurons. At the same time the firing rates and the modal interval values were also determined.

Some of the neurons were strongly activated in behavioral states associated with cortical desynchronization. For these cells the temporal relationship between the activation and the desynchronization was also investigated at the SWS-PS transitions. We found that this transition could be determined more exactly and unequivocally than other transitions between stages of sleep-wakefulness cycle. The first sign of cortical desynchronization was regarded as the beginning of the PS episode. The moment of the firing change was defined as the point where the cumulative discharge curve, computed and plotted from a 150 sec period around the transition, changed in slope. If more than one transition was analyzed for a given cell, the latencies were averaged.

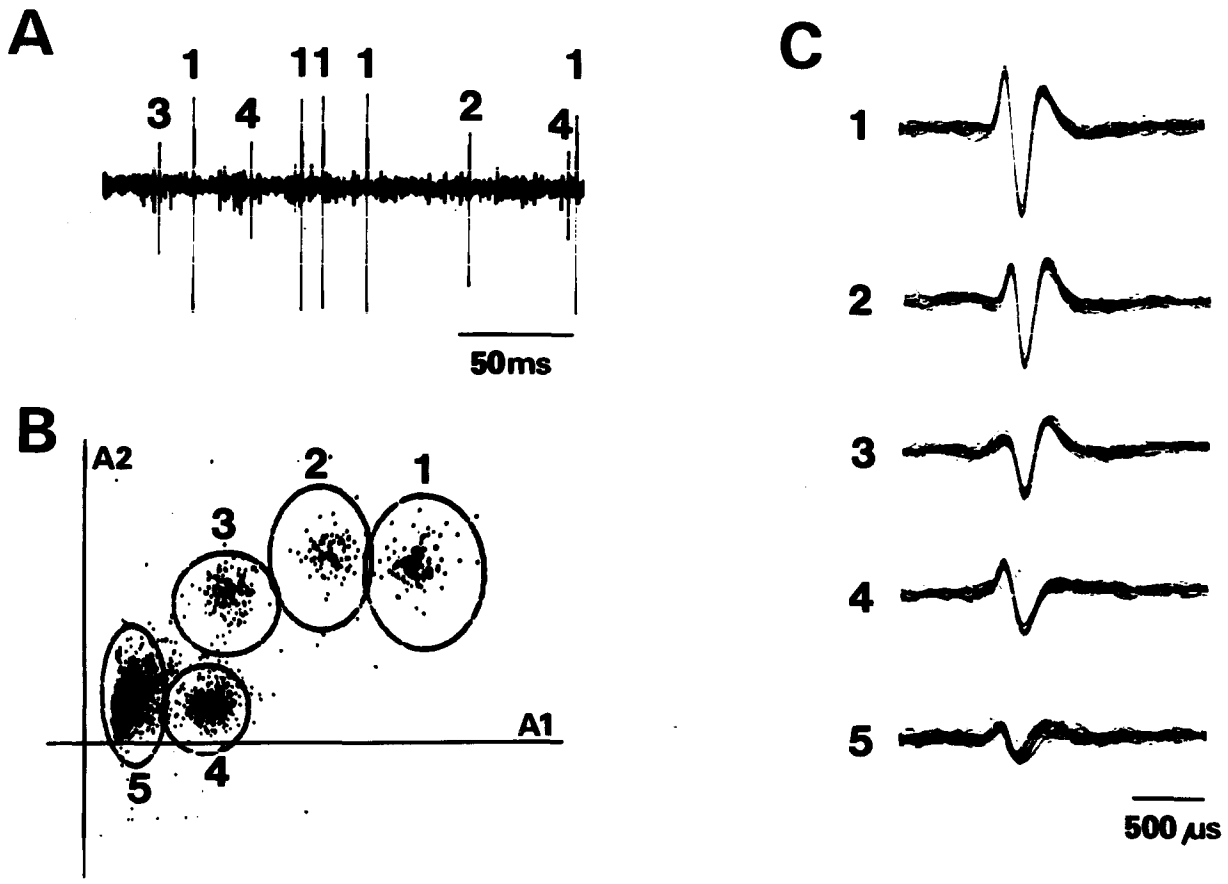

Fig. 1. Separation of differently shaped discharges recorded by the same electrode. A: a short example of the original record. The numbers refer to the clusters in B. B: 1 of the 3 plots (A1-A2, A1-DT, A2-DT) constructed from the 3 measured spike parameters: the amplitude of the first (A1) and second (A2) peaks and the time (DT) elapsing between them. The similarly shaped discharges form clusters which can be delimited with ellipses. C: using the equations of the ellipses, the spikes are separated and their variability checked by superimposing 10 of them on the screen of a storage oscilloscope.

Results

A total of 35 points were explored. Most were located in the border area of globus pallidus and putamen, but some were in various peripallidal structures (Fig. 2). The firing rates and patterns of 150 units were examined in the 3 phases of the sleep-wakefulness cycle.

Patterns of neuronal firing

On the basis of their spontaneous firing (Figs. 3 and 4), the neurons were divided into 5 groups.

(1) W-SWS-PS bursting type (12 cells). These neurons fired in short, high-frequency bursts (2–5 spikes, 300–400 c/sec) with long silent periods between them in all stages of the sleep-wakefulness cycle. The bursting pattern is reflected in the shape of autocorrelograms and in the short modal interval values (Figs. 3 and 4). The mean firing rate was relatively high in W and PS and decreased moderately during SWS. The cells were found exclusively in points within the GP and the border area of GP and putamen (Fig. 2).

(2) W-SWS-PS regular type (38 cells). The most striking feature of the neurons sorted into this group was the regular firing characterized by

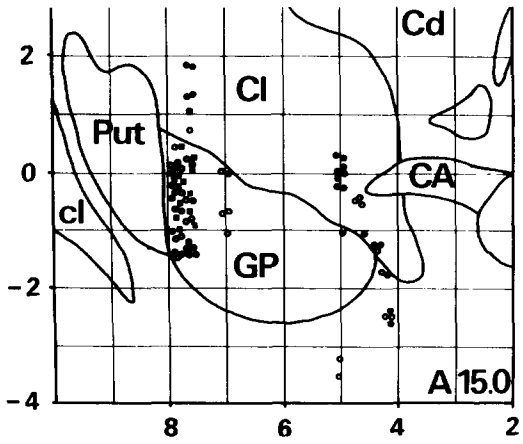

Fig. 2. Localization of the neurons sorted into 1 of the first 3 groups. Filled squares, W-SWS-PS bursting type; open circles, W-SWS-PS regular type; filled circles, W-PS regular, SWS bursting type. The cells belonging to the remaining 2 groups are not displayed.

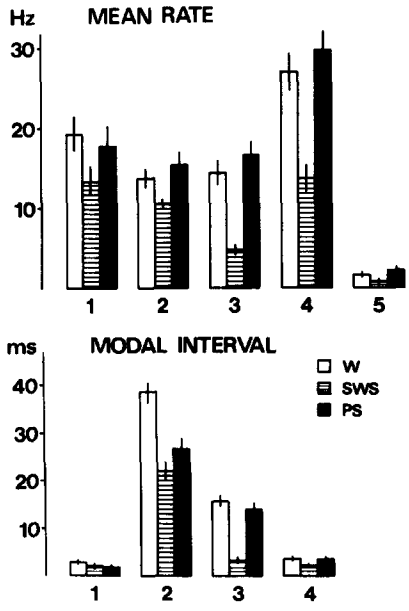

Fig. 3. Changes of mean firing rates and modal interval values in the 5 groups in the phases of sleep-wakefulness cycle. In each group the firing was significantly ($P < 0.05$ or < 0.01, Student t test) lower in SWS than in W or PS, except the SWS-PS comparison in group 1. Modal intervals also differed significantly across sleep-waking states, except in group 1. Comparing W to PS, neither the mean rates nor the modal intervals differed statistically. 1, W-SWS-PS bursting type; 2, W-SWS-PS regular type; 3, W-PS regular, SWS bursting type; 4, W-PS irregular, SWS bursting type; 5, W-SWS-PS sporadic type.

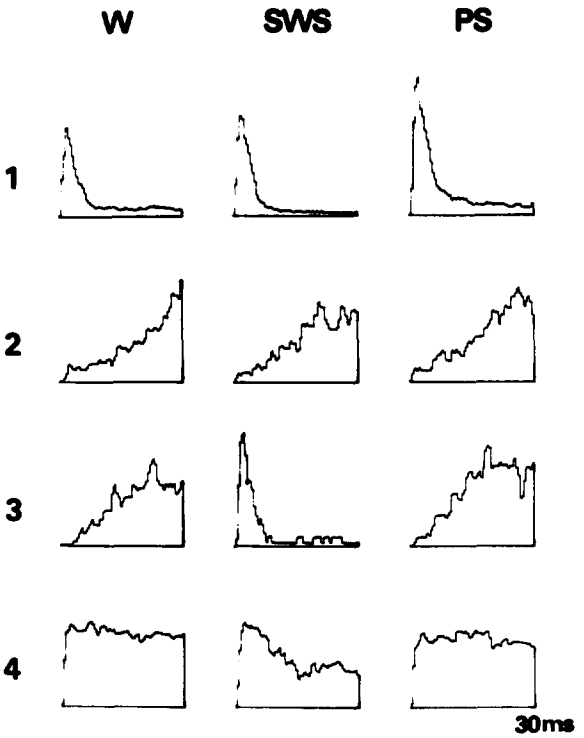

Fig. 4. Autocorrelograms of 4 neurons each representing the firing patterns of 1 of the first 4 groups in the different states of sleep-wakefulness cycle. Because of the low discharge rate, the autocorrelograms of group 5 neurons were practically empty at this bin-width and were omitted.

long modal intervals during both wakefulness and sleep. Accordingly, the firing probability rose slowly after a discharge as is clearly seen on the autocorrelograms (Fig. 4). The firing rate changed only moderately during the sleep-wakefulness cycle in this group, too. The average firing level was somewhat lower than in the former group (Fig. 3). Most of the cells were found at the border of GP and putamen, the others in the substantia innominata and in the vicinity of the comissura anterior (Fig. 2).

(3) W-PS regular, SWS bursting type (27 cells). The firing pattern of these neurons was a mixture of patterns observed in the first two groups. The cells discharged regularly in W and PS, but during SWS the mean rate decreased markedly and the spikes clustered in short, high-frequency bursts (Fig. 4). The regularity in W and PS was less

pronounced than in group 2: shorter modal interval values were measured at equal discharge levels (Fig. 3). The bursts generated by the cells during SWS resembled those of the neurons in the group 1, but appeared more sporadically; the mean rate was 4.8 c/sec in group 3 compared with 13.4 c/sec in group 1. There was a strong correlation between the level and pattern of firing: the bursting tendency appeared also in W, whenever the firing rate decreased. The discharges were recorded from points located at the border of GP and putamen, in the substantia innominata and dorsally from the GP in the capsula interna (Fig. 2).

(4) W-PS irregular, SWS bursting type (38 cells). These neurons were characterized during SWS by the occurrence of short bursts embedded in a stochastic background firing (Fig. 4). In some cases, the bursts were observed also in PS, but more generally, the activity was random following the Poisson distribution in W and PS. The modal intervals were below 4 msec. Comparing to other groups, the mean rates were higher, with marked decrease during SWS (Fig. 3). The anatomical localization of these cells (not shown) was similar to that of the group 3 neurons.

(5) W-SWS-PS sporadic type (35 cells). There was a distinct group of neurons discharging only sporadically in all 3 phases of sleep-wakefulness cycle (Fig. 3). Owing to the low number of spikes encountered during the 50 sec sampling periods, no clear-cut firing pattern was verified from the autocorrelograms. The firing rate was the lowest in SWS. For the anatomical localization the same is true as for group 4. As the low firing rates made any comparison uncertain, this group was not further analyzed.

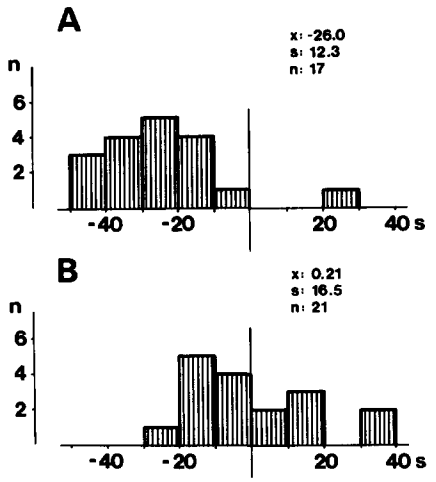

Fig. 5. Relationship between the neuronal and cortical activation at the SWS-PS transition in the neurons of group 3 (A) and group 4 (B). The columns display the distribution of latencies of the increased firing rates in relation to the onset of PS.

Individual modifications of firing rate

The firing rates of the individual neurons changed in most cases in accordance with the respective group averages during the sleep-wakefulness cycle (Table I). The majority of cells in 1, 3 and 4 groups was less active in SWS compared to W. However, a relatively large part of the group 2 cells either did not change or increased (13.2% and 15.8%, respectively) its firing rate slightly during SWS. Two (7.4%) out of the 27 members of the group 3 were also activated moderately during SWS.

Paradoxical sleep was accompanied by an increase of firing rates in all 4 groups compared to SWS, with only few exceptions in the group 1 and group 2 cells. The comparison of discharge levels

TABLE I

The percentages of cells of the different groups (explanation of numbers in Fig. 3) firing with higher, lower or equal frequencies in different sleep-wakefulness states. Only those differences exceeding an arbitrary limit of 10% were considered as higher or lower.

	SWS > W	SWS < W	SWS = W	PS > SWS	PS < SWS	PS = SWS	PS > W	PS < W	PS = W
1	–	91.7	8.3	81.8	9.1	9.1	18.2	27.3	54.5
2	15.8	71.0	13.2	86.1	8.3	5.6	52.8	16.7	30.5
3	7.4	92.6	–	100.0	–	–	59.3	14.8	25.9
4	–	100.0	–	100.0	–	–	48.7	16.2	35.1

in wakefulness and paradoxical sleep revealed less uniform differences. In the last 3 groups (2, 3 and 4) 50–60% of the units were more active during PS and only 15–16% were more active during W. In contrast, in the group 1 these percentages were: 18.2% and 27.3%, respectively.

Timing of firing changes at SWS-PS transitions

The neurons of groups 3 and 4 were strongly activated in behavioural states associated with cortical desynchronization (W, PS). For these neurons the timing of firing changes at SWS-PS transitions was also examined in every case when the transition was recorded in appropriate length: 18 out of 27 cells of group 3 and 21 out of 35 neurons of group 4 were analyzed (Figs. 5 and 6). All but one cell of group 3 were activated well before the onset of PS (Fig. 5A). The mean ± S.D. of latencies was 26.0 ± 12.3 sec. In those cases when PS developed through a short (20 sec) ill-defined period, the firing rate decreased again after an initial increase. At the end of this period a second, stronger activation was observed. In contrast to group 3, the latencies of neurons belonging to group 4 were distributed around zero with a mean ± S.D. of 0.21 ± 16.5 sec (Fig. 5B). Four cells even decreased their firing rate temporarily before the transition.

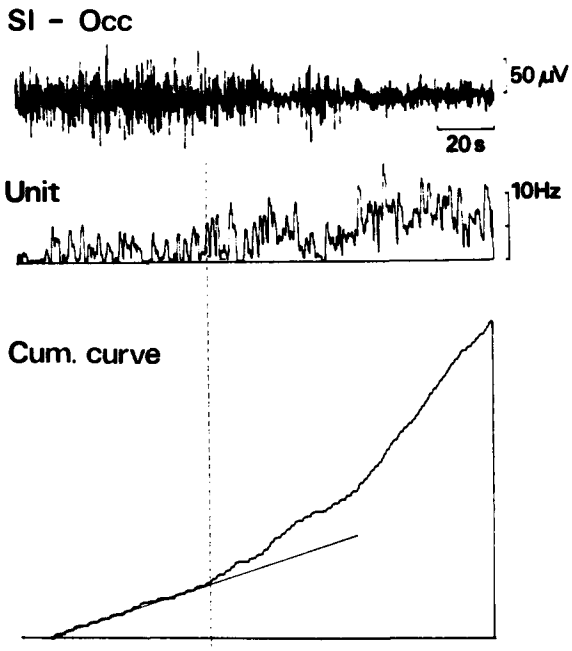

Fig. 6. Firing of a neuron belonging to group 3 at the SWS-PS transition. The beginning of the cortical desynchronization was regarded as the onset of PS. The momentary firing rate ('unit') was calculated in 150 msec/bin resolution. The dashed line depicts the point where the slope of the cumulative discharge curve begins to depart from the linear increase.

Discussion

Contrasting experimental evidence relates the BFA either to the regulation of slow-wave sleep (Sterman and Clemente 1963; Obal et al. 1980; Benedek et al. 1981), or to the control of low voltage fast activity (Stewart et al. 1984). Investigating the neuronal events occurring in the BFA during the sleep-wakefulness cycle, we examined previously the unit activity in the classical hypnogenic areas — POA and TO (Detari et al. 1984). The aim of the present study was the investigation of the pallidal region, where large neurons belonging to the basal forebrain cholinergic system (BFCS) were found (Kimura et al. 1981). The BFCS is claimed to be the only major source of cortical acetylcholine (Johnston et al. 1981), a transmitter believed to be related to cortical desynchronization (Vanderwolf and Robinson 1981).

Two neuronal groups (3 and 4) differing in their firing patterns were found to be strongly activated during W and PS, i.e., during behavioral states associated with cortical desynchronization. The analysis of the temporal relationships at SWS-PS transitions revealed that in one of these groups (group 3) the activation of neurons started well before any sign of desynchronization. This finding suggests that the enhanced firing of these neurons may be causally related to the cortical activation. Neurons activated in advance of cortical desynchronization have also been reported from the brain-stem reticular formation (Hobson et al. 1974; Steriade et al. 1982), which structure is also claimed to be the source of cortical activation during both W and PS (Steriade 1981). It may be supposed that the BFA neurons are excited by the reticular formation and that the increased activity is not locally generated. This assumption is supported by the existence of anatomical connections between

brain-stem structures and BFA (Nauta and Kuypers 1958; Shute and Lewis 1967; Woolf and Butcher 1986).

The investigated area contains large neurons belonging to the basal forebrain cholinergic system (Kimura et al. 1981). This system is probably strongly excited during cortical activation, as the ACh release from the cortex was shown to increase enormously during desynchronization (Szerb 1967; Jasper and Tessier 1971), and this ACh presumably originates from the basal forebrain neurons (Johnston et al. 1981). These facts raise the possibility that our group 3 neurons are identical with the cortically projecting cholinergic cells. However, no direct proof was obtained in the present study to support this assumption. The opposite assumption was made in a recent paper by Szymusiak and McGinty (1986) who tentatively identified the cholinergic cells with a group of neurons displaying much higher activity during SWS than W or PS. The discovery of the SWS-specific neurons is undoubtedly a very important step in the solution of the controversial role played by the BFA in the regulation of the sleep-wakefulness cycle. However, the assumption that these cells are cholinergic seems to contradict the evidence indicating cholinergic participation in cortical activation. An unequivocal resolution of this controversy would be the antidromic identification of the neurons from the cortex, since it was shown that the cortically projecting BFA cells are almost exclusively cholinergic (Wahle et al. 1984). This task, however, seems to be very difficult to accomplish in freely moving animals, because the individual cholinergic cells have only restricted cortical projections (Price and Stern 1983; Saper 1984).

A further, indirect indication of the cholinergic nature of the group 3 cells might be the regular firing pattern, which seems to be characteristic for the cholinergic BFA cells in the waking state (DeLong 1971; Richardson and Thompson 1985). Our group 3 neurons fired regularly in W and PS, and even the firing rates and modal interval values were in good agreement with those described in the above mentioned papers on monkeys and rabbits. However, the group 2 neurons also discharged regularly during both cortical desynchronization and SWS. The firing rate of these cells changed only moderately in the course of the sleep-wakefulness cycle, which fact is inconsistent with the changes of cortical ACh release (Szerb 1967). Therefore, the regular firing alone does not seem to be a reliable criterion for the identification of the cholinergic BFA neurons.

To summarize, the neurons of group 3 are closely related to cortical desynchronization. On the basis of their anatomical localization, firing changes and firing pattern, it may be assumed that they are identical with the cortically projecting cholinergic cells and that they transfer the influences of brain-stem structures to cortical areas. However, this assumption needs further and unequivocal confirmation.

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