Changes of Simple and Complex Spike Activity of Cerebellar Purkinje Cells with Sleep and Waking

Abstract. *Action potentials of cerebellar Purkinje cells were observed in intact monkeys during sleep and waking. Purkinje cells exhibit two sorts of action potentials, called simple and complex spikes, and these two sorts of spikes were differently affected by sleep. Simple-spike activity (generated by the parallel fiber inputs to the Purkinje cell) was highest during sleep with rapid eye movements as compared with both waking and sleep with electroencephalographic slow waves. In contrast, complex-spike activity (generated by the climbing fiber inputs to the Purkinje cell) was lowest during sleep with rapid eye movements. The complex action potential of the Purkinje cell consists of an initial large spike followed by one or more smaller secondary spikes, and the number of these secondary spikes was found to be independent of the background discharge frequency of the simple spike. This independence suggests a possible role of presynaptic factors rather than the excitability level of the Purkinje cell itself in determining the number of secondary discharges occurring in the complex spike.*

The cerebellar Purkinje cell is excited by two classes of inputs: a parallel fiber input (which generates a simple spike) and a climbing fiber input (which generates a complex spike) (*1–3*). The configuration of these two spikes is strikingly different, enabling one to distinguish between them even with extracellular recordings (*4*). Studies of Purkinje cell activity in intact monkeys have shown extreme differences in discharge frequencies of simple spikes (SS) as compared with complex spikes (CS). Thach (*4*) observed average frequencies of 70 per second for SS as compared with about 1 per second for the CS. Another difference between SS and CS was seen during movement: Thach found obvious relations between SS activity and movement, whereas the relation between movement and CS discharge was considerably more elusive. The functional role of the climbing fiber input to the Purkinje cell, and the CS which this input elicits, are thus matters of considerable uncertainty. One of the most intriguing ideas as to the role of the climbing fiber input was put forward by Brindley (*5*) and elaborated by Marr (*6*). Marr suggested that the climbing fiber has a role in learning, and that ". . . if a parallel fibre is active at about the same time as the climbing fibre to a Purkinje cell with which that parallel fibre makes synaptic contact, then the efficacy of that synapse is increased towards some fixed maximum value." Studies of CS activity of Purkinje cells in animals capable of carrying out learned, voluntary movement may provide data useful in pursuing the hypothesis of a role of climbing fiber inputs in learning, and the present study on CS activity recorded from intact monkeys was undertaken to obtain baseline data as a background for subsequent studies of CS activity during acquisition of motor skills.

Extracellular records of Purkinje cell activity were obtained with microelectrodes as previously described (*4, 7*). In addition, macroelectrodes were used to record electroencephalograms, electrooculograms, and electromyograms in order to identify waking, sleep with electroencephalographic slow waves (S-SW), and sleep with rapid eye movement (S-REM). The criteria for the identification of Purkinje cells were the same as those adopted by Thach (*4*). Spontaneous firing of 84 such cells was recorded in three monkeys (*Macaca mulatta*). Of these 84 cells, eight were observed during repeated episodes of waking, S-SW, and S-REM. It is for these eight Purkinje cells that results on S-REM are presented in this report. Units were often injured or lost during the episodes of S-REM, presumably owing to variation of intracranial blood flow or blood pressure (*8*). It was found that the instability produced by these vascular effects was greatly reduced in lateral portions of cerebellar hemispheres as compared to midline structures, and the eight stable Purkinje cells described in this report were all located in the lateral portion (12 to 17 mm lateral to the midline of the cerebellum) of lobulus simplex (VI) and ansiform lobule (VIIA).

The mean firing rate for the SS of eight Purkinje cells during waking, S-SW, and S-REM is presented in Fig. 1A. The change from waking to S-SW was negligible, but there was a striking increase of SS frequency from S-SW to S-REM: discharge frequency rose in each of the eight units, and for the group as a whole the mean rose from 26.2 during S-SW to 49.4 during S-REM. Interspike interval distributions showed a similar change: the percentage of interspike intervals less than 10 msec was eight in waking, two in S-SW, and 24 in S-REM; the percentage of intervals greater than 50 msec was 22 in waking, 28 in S-SW, and 11 in S-REM. From these analyses it can be concluded that SS activity of the Purkinje cell is increased in S-REM as compared with either waking or S-SW.

Data on CS activity of Purkinje cells were obtained by examination of photographic records to determine the mean firing rate of the CS and the number of secondary spike discharges

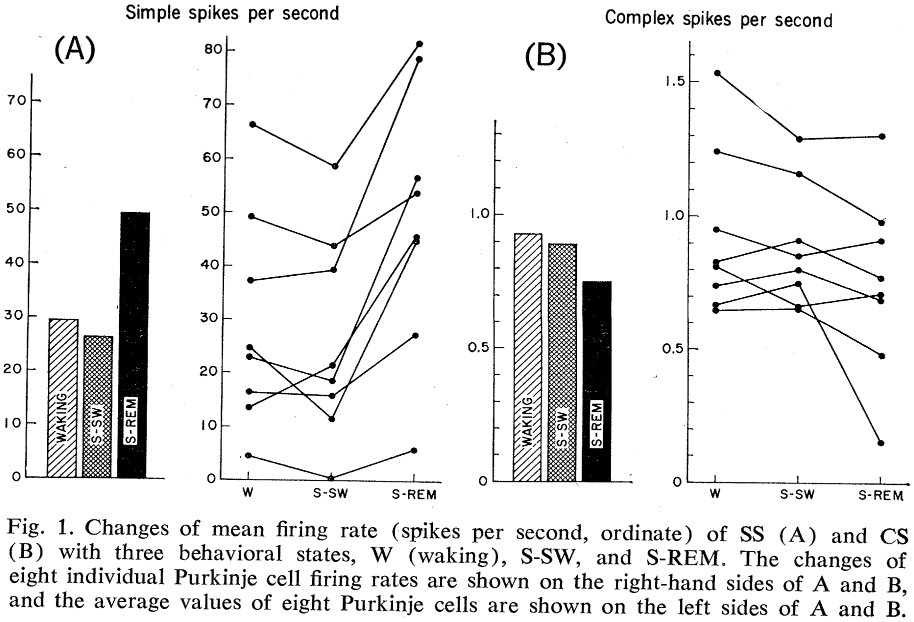


Fig. 1. Changes of mean firing rate (spikes per second, ordinate) of SS (A) and CS (B) with three behavioral states, W (waking), S-SW, and S-REM. The changes of eight individual Purkinje cell firing rates are shown on the right-hand sides of A and B, and the average values of eight Purkinje cells are shown on the left sides of A and B.

associated with each individual CS. The mean firing rates of CS are shown in Fig. 1B. The effect of S-REM on firing rates of CS was opposite to that seen in the case of the SS. The average firing rate of the CS for the eight units showed a minimum value of 0.75 per second during S-REM, compared with 0.93 per second during waking and 0.89 per second during S-SW. Though the change in mean discharge frequency of the CS was slight, it should be noted that the reduction from waking to S-REM occurred in all eight units. Thus, for each and every unit the SS was most active in S-REM, whereas the CS of each of the same eight Purkinje cells changed in the opposite direction, being least active in S-REM.

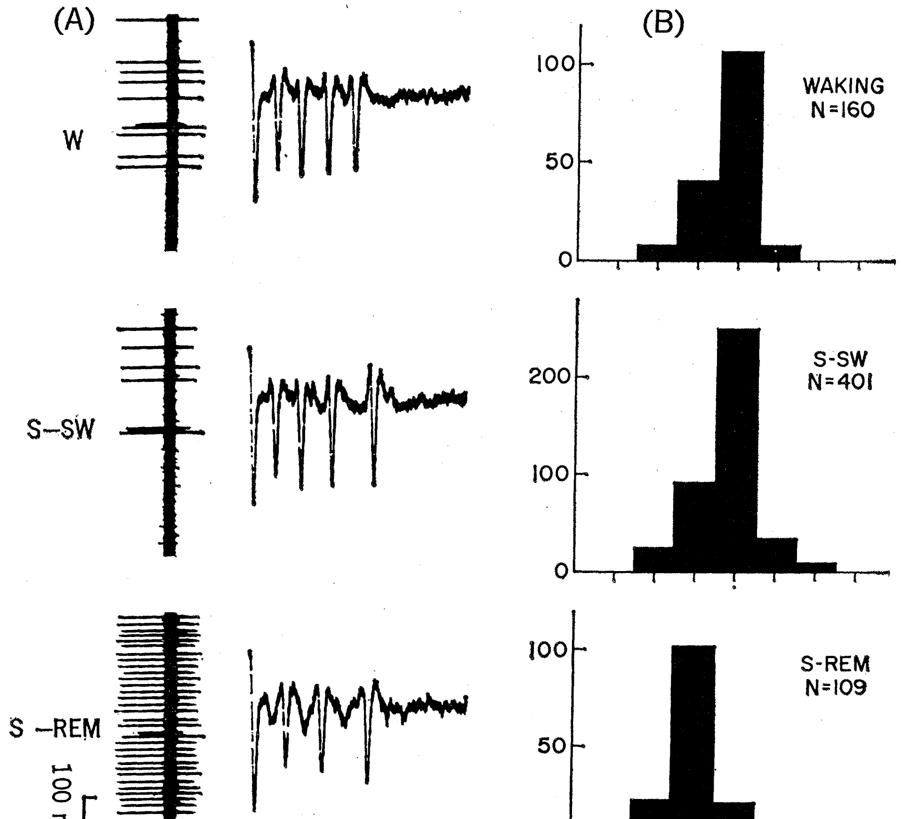
In the above analysis of CS firing rate, each CS was treated as a unitary event, but actually each CS is composed of an initial spike followed by a short burst of one or more secondary spikes. Therefore, to describe the CS it is necessary to observe its detailed configuration. From photographic records, as is shown in Fig. 2A, it was possible to count the number of secondary spikes in each CS throughout the three behavioral states. This was done by triggering the oscillograph sweep on the large initial spike of the CS and counting the subsequent secondary spikes on photographic records. Such triggering could be done reliably in five of the eight Purkinje cells, and it is for these five cells that the number of secondary waves was counted during waking, S-SW, and S-REM. Figure 2B shows the distribution of the number of CS secondary discharges for one Purkinje cell. The distributions during waking and S-SW were almost the same; more than 60 percent of CS were composed of an initial spike followed by four secondary spikes. In contrast, during S-REM the distribution was shifted toward lower numbers, that is, about 70 percent of CS had three secondary spikes. In four of the five units similar changes, that is, a shift to lower numbers, were observed in the distribution of secondary discharges of CS. In only one unit a slight shift toward higher numbers was observed.

Table 1. Simple spike frequency (impulses per second) prior to complex spikes with two or four secondary waves.

Two secondary waves*	Four secondary waves*
93	98
21	36
26	23
90	66
49	54
25	40
70	58
9	16
70	59

* The mean for nine Purkinje cells is 50.

A different type of analysis was carried out to discover whether the number of secondary waves on a CS might vary depending on the intensity of SS activity occurring immediately prior to the CS. In this analysis the discharge frequency of SS was determined during the 125 msec immediately prior to the CS, and the frequency of SS discharge was examined in relation to the number of secondary waves of the CS. From this analysis, carried out for



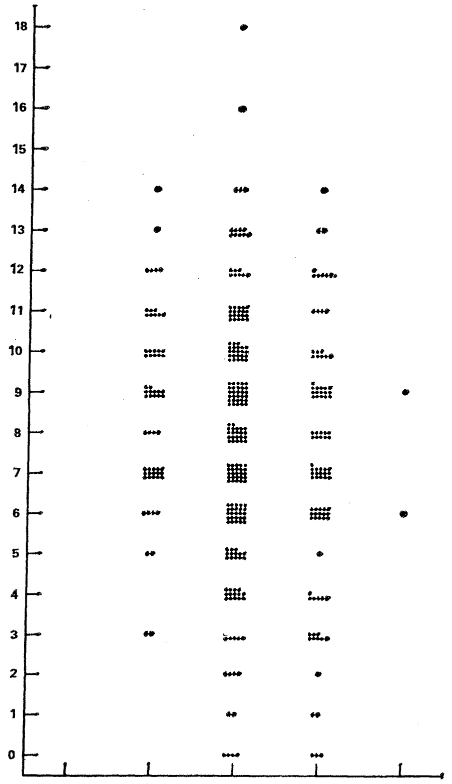


Fig. 2 (left). Changes of configuration of CS during waking (W), S-SW, and S-REM. (A) Specimen photographic records of CS with its background activity of SS before and after the CS. Time runs from bottom to top and from left to right in slow- and fast-sweep photographs, respectively. The fast oscillographic sweep was triggered selectively by larger positivity of initial spike of the CS. Positivity is rightward and upward in slow and fast sweep, respectively. (B) The distribution of number of secondary spikes (abscissa) in each of CS (number of CS in ordinate) in three states from the same unit as is shown at left. Fig. 3 (right). A specimen scatter diagram of one Purkinje cell unit showing the independence of the number of secondary spikes in a CS (abscissa) from the number of SS during 125 msec immediately prior to the CS (ordinate).

nine Purkinje cells during waking (Fig. 3 and Table 1), it was found that the number of secondary waves was entirely independent of the immediately preceding activity of the SS. This observation is inconsistent with the hypothesis of Eccles *et al.* (*2, 9*) that the number of secondary waves reflects the level of Purkinje cell excitability, and suggests a more important role of presynaptic factors (*10*) in determining the number of secondary spikes occurring in each CS.

Marchesi and Strata (*11*) recently reported the observation of Purkinje cell activity in the intact cat in relation to sleep and waking. Changes found in SS activity in S-REM in the present study are essentially the same as their report for the cat, and are also consistent with other reports on several other parts of the central nervous system (*12*). It is of note that SS activity represents most of Purkinje cell activity and that the Purkinje cell is the first indentified inhibitory neuron (*13*) in the central nervous system in which the highest activity during S-REM has been observed.

As to the CS activity, however, there are some differences between the report of Marchesi and Strata in the cat and the present results in the monkey. Marchesi and Strata reported higher firing rates of CS during desynchronized sleep when the rapid eye movements were absent, and occasional lower firing rates when they were present in desynchronized sleep, than the rates during S-SW. In the present study it was not possible to find any relation between the firing rate of CS and the existence of phasic events such as the rapid eye movement or muscle twitch. Whether these different observations are due to a difference in the sleep patterns of different species or due to some other factors cannot be assessed at the present time.

NORI-ICHI MANO*
Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Maryland 20014

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* Guest worker from the Department of Neurophysiology, Institute of Brain Research, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

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Improvement of Learning in the Aged by Modification of Autonomic Nervous System Activity

Abstract. *Partial blockade of beta-adrenergic end-organ response to the autonomic nervous system was effected in a group of older men by administration of propranolol. The result was improved performance in a learning task. The data support the hypothesis that the learning decrement found among older men is not simply a manifestation of structural change in the central nervous system but is, at least in part, associated with the heightened arousal of the autonomic nervous system that accompanies the learning task.*

Studies of verbal learning in older men have consistently demonstrated a prominent decrement in performance with advancing age, presumably indicative of a decline in a higher order cognitive functioning. Increasing evidence indicates, however, that such a decline cannot be attributed solely to the structural changes in the central nervous system that are known to accompany aging (*1*). Instead, differences in learning performance appear related, in part, to the failure of older persons to respond where rapid response is required. In situations where the pace of a learning task is slowed, the older person improves in performance significantly and responds relatively rapidly (*2–6*). Another age-related difference found in the learning situation is the more pronounced extent and persistence of plasma free fatty acid (FFA) mobilization among older subjects than among younger controls (*7*). It has been demonstrated that this age-related physiologic response is not simply a difference in ability to metabolize plasma FFA or to respond to infused catecholamine (*8*). Further, with plasma FFA mobilization again used as an indicator of autonomic arousal, evidence of a curvilinear relationship between autonomic activation and learning task performance was found (*4*). Therefore, it has been suggested that the heightened and prolonged autonomic arousal found during learning task performance in the older person is directly implicated in the tendency of older persons to commit more errors of omission, indicative of response suppression, in the rapidly paced learning situation (*9*).

Although the autonomic arousal found in conjunction with the learning task might merely reflect activation of the central nervous system associated with cognitive functioning, feedback from the peripheral manifestations of this arousal might, in itself, actively contribute to performance decrement. If this were the case in older persons, the masking of such autonomic effects should result in improvement in learning scores.

Propranolol (Inderal), by producing partial blockade of autonomic beta-adrenergic receptor sites in peripheral end organs, largely mitigates most physiologic concomitants of central nervous system arousal. Although small amounts of the drug might cross the "blood-brain barrier" (*10*), there is no evidence of resulting central nervous system activation or deactivation. With the use of this drug, it is therefore possible to test our hypothesis about the influence of autonomic arousal on learning in older persons. The impact of propranolol on learning performance could be interpreted as the effect of partial blockade of autonomic end-organ response. As a monitor of the drug effect, all subjects could be assessed for autonomic reactivity, with heart rate, plasma FFA level, and galvanic skin response serving as indices of physiologic arousal.

For this study the subjects were 28 paid male volunteers, 60 years of age or older (mean age, 68.6 years; range, 60