ELECTRICAL ACTIVITY OBSERVED IN VITRO IN THIN SECTIONS FROM GUINEA-PIG CEREBELLUM

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Summary Single cell discharges were recorded in vitro from thin sections of cerebellum of the guinea-pig prepared vertical to the cortical surface and incubated in artificial media. White matter stimulation induced antidromic, mossy and climbing fiber responses in neurons in the Purkinje cell layer. Spontaneous discharges were observed in both Purkinje cell and granular layers. In the Purkinje cell layer, most neurons repeated a regular cycle of firing and silent periods. Response of each neuron to white matter stimulation increased and decreased synchronously with this. At the transition from the firing to silent period, many cells generated a sequence of spike bursts. Application of glutamate also caused a similar burst sequence. A train of stimuli to the white matter was followed by a sustained suppression of the spontaneous discharge for several seconds. A single stimulation did not induce a long and profound suppression of spontaneous discharge. These findings were discussed in comparison with those obtained in vivo.

In response to electrical stimulation, thin sections taken from various parts of the mammalian brain generate relatively large field potentials and single cell discharges in artificial media (Kawai and Yamamoto, 1969; Richards and Sercombe, 1968; Yamamoto, 1972; Yamamoto and McIlwain, 1966). It has been rarely possible, however, to record spontaneous activity from thin brain sections as far as Ca$^{+}$+ concentration is kept in the physiological range (2.4 mM). The lack of spontaneous activity may be due to absence of continuous afferent input. Recently, the cerebellar Purkinje cells have been found to produce spontaneous bioelectrical activity even when they have been chronically deafferented or long cultured in vitro (Eccles et al., 1966a; Gähwiler et al., 1973; Schlapfer et al., 1972; Snider et al., 1967). These findings suggest that cerebellar neurons are active without afferent inflow. The purpose of the present experiments is to
examine whether neurons in freshly prepared cerebellar sections generate spontaneouss activity, and if results are confirmative, to elucidate some properties of the activity.

During preparation of this article, OKAMOTO and QUASTEL (1973) reported in a preliminary communication that neurons in cerebellar sections prepared parallel to the surface generated spontaneous discharges. Whereas their report is mainly concerned with biochemical aspects of the activity, the present communication deals with its physiological ones. Further, sections used in the present experiments seem more useful in future experiments, because they were prepared vertical to the cortical surface and therefore the white matter could be stimulated for anti- and orthodromic activations of neurons.

METHODS

Guinea pigs were stunned by a blow on the back of the neck and killed by a second blow on the back of the thorax. After removal of the scalp, the occipital bones were prized away with scissors. The cerebellum and underlying medulla oblongata were taken out of the skull. With a triangular knife made from a razor blade, the vermis was isolated (Fig. 1a) and placed on a piece of filter paper covered with the standard medium. Blocks of the cerebellar cortex of about 1 mm thick were prepared parallel to the surface of the folium with a razor blade (Fig. 1b) and then they were cut to a thickness of 0.25 mm at right angles to the long axis of the folium, either manually or with the aid of the Vibrotome (Oxford Laboratories, California, USA). When cut manually, blocks were immediately placed under a low power microscope and sectioned with a razor blade. When the Vibrotome was used, blocks were maintained in the ice-cold standard medium for 3–30 min until they adhered one by one to a small metal dish in such a manner that the long axis of the folium stood vertical to the bottom of the dish (Fig. 1c). Aron-Alpha (Toa Gosei Kagaku, Tokyo), alpha-acrylate monomer, was used as the adhesive which polymerized instantaneously when mixed with water. The block was supported by an agar block which also adhered to the dish (Fig. 1c, A). The dish was filled with the chilled standard medium and cooled from the outside by ice water in which the dish was partly immersed. Then, the block was cut...
with a razor blade (Fig. 1c, R) which proceeded slowly in the direction of the arrow in Fig. 1c while vibrating transversely at 50 cps. The sections prepared with the Vibrotome gave results identical with those prepared manually and, because of constancy in thickness, were used in most experiments. The sections were incubated in the standard medium for more than 45 min at 37°C and then transferred one by one into the observation vessel which was previously described in detail (YAMAMOTO, 1972). The vessel was continuously perfused with the standard or a modified medium. The composition of the standard medium was (mm, final concentrations); NaCl, 124; KCl, 5; KH₂PO₄, 1.24; MgSO₄, 1.3; CaCl₂, 2.4; NaHCO₃, 26; and glucose, 10.

Single cell discharges were recorded generally with glass pipettes of 2–3 μm tip diameter filled with 4 M NaCl. In some experiments, in which unit discharges were picked up in the granular layer, pipettes of 1 μm tip diameter filled with 2 M K-citrate were used. For stimulation of the white matter, a pair of silver wires, each of diameter 0.2 mm, were sharpened electrolytically, insulated to bare tips and stuck together side by side with a tip separation of 0.2 mm. The stimulus pulses were 0.2 msec in duration and 1–6 V in strength. The electrodes were inserted into the Purkinje cell or granular layer which could be identified with the aid of a low-power microscope.

RESULTS

Responses to white matter stimulation

It has been described in vivo that Purkinje cells show three characteristic responses to white matter stimulation: mossy fiber, climbing fiber and antidromic responses (ECCLES et al., 1967; MURPHY et al., 1973). In thin cerebellar sections too, a microelectrode inserted into the Purkinje cell layer encountered many cells which showed one, two or all of these responses. In favourable neurons, the three responses could be differentiated by adjusting stimulus strength. In the neuron in Fig. 2, weakest stimulation evoked the mossy fiber response with a latency of about 3.5 msec (record 1). A slightly stronger stimulus elicited, with a shorter latency, a complex spike train which appeared to correspond to the climbing fiber response (record 2). Further increase in strength resulted in generation of antidromic spike with a latency of less than 0.5 msec which was followed by the climbing fiber response (record 3). All these changes in response pattern occurred in an all-or-nothing manner with definite differences in threshold. Sometimes, the mossy fiber response was followed by a long train of spikes (Fig. 4a). Individual spikes composing the climbing fiber response were not distinct in some neurons, especially when the spike contour was biphasic with a prominent positive phase (Fig. 8a-2). Since recording electrodes were inserted in the close vicinity of the stimulated site, stimulus artifact was sometimes so large that identification of the Purkinje cell by antidromic activation was difficult. From the characteristic
Fig. 2. Responses of a Purkinje cell to white matter stimulation. Records 1, 2, and 3; elicited at stimulus intensities of 1.8, 2.0, and 2.2 in arbitrary unit, respectively. Record 1 was taken with higher amplification. In this and following illustrations, upward deflections imply positive polarity.

spontaneous discharge as mentioned below, however, most of the neurons encountered in the Purkinje cell layer were inferred to be purkinje cells.

**Spontaneous discharge**

Neurons in the Purkinje cell layer discharged at frequencies of 20 to more than 150 cps in the absence of any stimulation applied deliberately. The spontaneous discharge was commonly observed in the cells remote from the recording electrode as judged from small amplitude and purely negative shape of their spikes. The spontaneous discharges as well as the responses to white matter stimulation were consistently observed in almost all experiments at least for several hours after preparation of the tissue. Spikes recorded extracellularly did not exceed 5 mV in amplitude. Further advance of the electrode caused penetration of the cell membrane. Although some cells discharged continuously and almost regularly, firing of most cells started abruptly, sustained for several seconds to minutes, ceased for 5 to 30 sec and then started again (Fig. 3). This is consistent with the observation made in vivo that spontaneous firing occurred in blocks lasting from a few seconds to several minutes (Bell and Grimm, 1969). The durations of the firing and silent periods varied from cell to cell, but they were roughly constant in each cell as far as the contour and size of spikes remained unchanged. Apparently, individual cells started and ceased to fire independently of other cells. In Fig. 3, in which spikes of three different cells were recorded simultaneously, the cell of middle spike size commenced to fire during (record 3), immediately after (record 2) or having no relation to discharge of the cell of the largest spike height (record 1).

When test stimulus was given at a low frequency to the white matter, it was found that the response of the cell was minimum at the beginning of the silent period and gradually increased toward the end of the firing period. In the cell in Fig. 4, a test shock at a later stage of the silent period elicited a spike train lasting about 0.6 sec (record a). The spike train evoked 4.2 sec later was higher
Fig. 3. Spontaneous discharges of three neurons in the Purkinje cell layer. Intervals between records 1 and 2, and between records 2 and 3 were about 1.5 sec each.

Fig. 4. Responses to white matter stimulation during firing and silent periods. Recorded from a neuron in the Purkinje cell layer. Records in column 2 were taken with expanded sweep. Dots indicate instances of stimulation. Intervals between successive records were 4.2 sec each, except those between c and d, and between e and f which were 2.1 sec each.

in frequency (record b). Then, without interruption, the firing period began during which excitability of the cell was so elevated that the same test shock induced a pause of firing due to hyperdepolarization reported by GRANIT and PHILLIPS.
(1956) (records d and e). As shown in expanded record d2, this consisted of an initial spike of almost normal amplitude followed by a burst of abortive spikes occurring at high frequencies. Immediately after the beginning of the silent period, the test shock evoked a spike train as short as 0.13 sec in duration (record f). The spike train was gradually prolonged (record g) until it was obscured by discharges occurring during next firing period.

In about a half of 120 cells studied, the firing period ended with repeated burst discharges. The cell in Fig. 5A which discharged at about 120 cps

![Fig. 5. Sequences of spike bursts at transition from the firing to silent period. Recorded in the Purkinje cell layer. In A, records 2, 3, 4, and 5 were taken 5, 25, 40, and 45 sec after record 1, respectively; B, recorded from another neuron.](image)

at the onset of the firing period (record 2), gradually increased the discharge frequency (record 3) and then started to generate a succession of spike bursts. Each burst was about 100 msec in duration and was composed of 10 to 20 spikes which rapidly decreased in size and reversed in polarity at the end of the burst, being transformed into a short positive spikes (record 4). Finally, the cell ceased to fire (record 5). As discussed later, the rapid reduction in spike height during the burst was probably due to an excessive depolarization of the cell. The silent period, however, does not seem due to spike inactivation, because spikes of high amplitude could be evoked by white matter stimulation throughout the silent period as shown in Fig. 4. It was also often observed that the burst sequence was not immediately followed by the silent period, but a spike train occurring at lower frequency than during the burst intervened between the end
of burst sequence and the onset of silent period (Fig. 5B). This also suggests that the silent period was not brought about by spike inactivation.

When electrodes were inserted into the granular layer, many small spikes were found to occur spontaneously as background noise. But, it was difficult to isolate single spikes, probably because of small size of the granule cells. In only a few cells spikes were well isolated as shown in Fig. 6. This cell responded to white matter stimulation with a train of spikes (record 1). In contrast to relatively regular firing of the neurons in the Purkinje cell layer, this cell discharged sporadically in a burst (record 2).

**Effects of glutamate**

If the burst sequences in Figs. 4d and 5A-4 were due to excessive depolarization, it would be expected that similar phenomena may take place, when glutamate, an excitatory amino acid (CURTIS and WATKINS, 1963), was applied in a high concentration. In the experiment of Fig. 7, sodium glutamate was applied in the perfusing medium at a concentration of 0.3 mm. Within 1 min of glutamate administration, the discharge frequency markedly increased (record 2). Subsequently, spikes decreased in height and occurred in bursts of short duration (record 3). Immediately after the standard medium was introduced into the

![](image)

**Fig. 7.** Effects of glutamate. 1, control record; 2 and 3, 1 and 4 min after 0.3 mm sodium glutamate, respectively; 4 and 5, 0.5 and 8 min after washing the section with standard medium, respectively.
observation chamber, the cell’s firing completely disappeared (record 4) and gradually resumed in a time course of about 2 min (record 5). It remains to be studied what mechanism caused the complete silence of the cell after glutamate. A possible explanation is that the silence was due to hyperpolarization caused by an electrogenic sodium-pump (Nakajima and Takahashi, 1966) which excreted Na⁺ accumulated in the cell under the action of glutamate.

**Suppression of spontaneous discharge**

Eccles et al. (1966b) reported that juxtafastigial stimulation induced in vivo a large inhibitory postsynaptic potential (IPSP) of long duration in the Purkinje cells. In sections used in the present experiments, a single shock to the white matter failed to evoke a long and profound suppression of the spontaneous discharge corresponding to the IPSP reported by Eccles et al. (1966b). On the other hand, the spontaneous discharge of the Purkinje cells was suppressed for more than several seconds by repetitive stimulation of the white matter. The cell in Fig. 8 was identified as the Purkinje cell by antidromic activation (record a-1).

![Fig. 8. Suppression of spontaneous discharge by white matter stimulation. Recorded from a Purkinje cell. a, superimposed traces; a-1, antidromic firing; a-2, climbing fiber responses induced by higher stimuli; a-3, recorded with slower sweep after stimulus strength was further increased by 20%; b and c, suppression by short pulse trains at 30 cps. Stimulus intensity was same with that used in a-2. Record b-2 was taken after a break of 3 sec. Records c-2 and -3 were taken after breaks of 5 sec each. Dots indicate stimuli.](image)

and also by the climbing fiber response induced by stronger stimulation (record a-2). This cell discharged regularly at 60 cps without cyclic changes in firing pattern. A short pulse train at 30 cps decreased the firing frequency for about 2 sec (record b). An increase in pulse number resulted in a complete suppression of the discharge sustaining for about 10 sec (record c). Though in these records the suppression started 0.1 sec after the stimulus train was turned off, further increase in pulse number or in pulse intensity shortened the interval between the end of the pulse train and onset of the suppression. The prolonged suppression of the spontane-
ous discharge was probably not caused by the inactivation of the spike generation, because spikes induced by test shock during the suppression showed no indication of inactivation such as reduction in height or change in configuration. In contrast with such a long suppression induced by pulse train, a single shock suppressed the spontaneous discharge only for 25 msec after the end of the climbing fiber response even though stronger stimulation was used (record a-3).

**DISCUSSION**

Recently, HACKETT (1972) reported that the cerebellum of the frog isolated and maintained in vitro generated the antidromic, mossy as well as climbing fiber responses. In the present experiments, thin sections from the mammalian cerebellum were also shown to produce all these characteristic responses. Although it was observed in vivo in the cat cerebellum (GRANIT and PHILLIPS, 1956) that spikes recorded extracellularly were very large (commonly 20–40 mV amplitude), spike height did not exceed 5 mV in the present experiments. The difference may be attributed to larger electrode tips in these experiments or to diversity of species.

Neurons in cerebellar sections discharged spontaneously. When spikes were positive in polarity, the spontaneous firing might be partly due to mechanical injury by electrodes (ECCLES et al., 1967). The fact that spontaneous discharges were recorded from neurons remote from electrode tips as inferred from their small and purely negative spikes, however, indicates that the spontaneous discharge is not attributable to the mechanical injury alone. The presence of spontaneous activity in the chronically isolated cerebellum (BROOKHART et al., 1950; ECCLES et al., 1967; SNIDER et al., 1967), in tissue culture (GÄHWILER et al., 1973; SCHLAPFER et al., 1972) and in cerebellar sections suggests that either the Purkinje cells have an inherent ability to generate spontaneous activity or they are under continuous activation from granule cells. GÄHWILER et al. (1973) in tissue culture, and OKAMOTO and QUASTEL (1973) in cerebellar sections showed that the spontaneous discharge persisted, though decreased in frequency, in the presence of Mg++ at high concentrations enough to block synaptic transmission. Consequently, it may be inferred that the Purkinje cells can generate action potentials without synaptic activation. In the physiological concentrations of Mg++ and Ca++, synaptic activation from granule cells may also contribute to generate spontaneous activity in the Purkinje cells. This is suggested by presence of spontaneous activity in the granular layer.

According to ECCLES et al. (1966b), juxtafastigial stimulation induced a large IPSP of long duration in the Purkinje cells in anesthetized cats. They considered that the inhibition is mediated by successive activation of granule and basket cells. In the present experiments, a single shock to the white matter did not induce such a profound suppression of the spontaneous activity as expected from their observation. In the sections used, granule cell axons were cut in a length of 250 µ and
therefore a considerable part of axons was presumably inactive because of damage. The lack of the basket cell inhibition, therefore, may partly be explained by inefficiency of damaged granule cell axons to activate basket cells. The observation that many cells in the Purkinje cell layer responded to white matter stimulation with mossy fiber response, however, indicates that granule cells were still powerful in activating neurons making synaptic contacts with them. Therefore, the partial damage to granule cell axons during preparation of the tissue cannot be the main cause of the lack of basket cell inhibition. In this connection, the finding by Bloedel and Roberts (1969) is worthwhile noting that the surface stimulation, which exerted a powerful inhibitory action on the Purkinje cells in anesthetized cats, had no inhibitory effects in unanesthetized cats. They explained this phenomenon by the assumption that spontaneous firing of Purkinje cells continuously inhibited basket cells in unanesthetized cats and therefore, the number of basket cells activated by surface stimulation was too small to induce a powerful inhibition. A decrease in firing frequency of the Purkinje cells by anesthesia was thought to release basket cells from the continuous inhibition, thereby allowing surface stimulation to recruit a larger number of basket cells. The same explanation may apply to the lack of the inhibition in the present experiments. The explanation of Bloedel and Roberts (1969), however, still remains to be examined in future studies because Murphy and Sabah (1970) did not find statistically significant differences in terms of Purkinje cell firing frequencies between anesthetized and unanesthetized cats.

In contrast to the lack of basket cell inhibition, a long suppression of the spontaneous discharge took place after repetitive stimulation of the white matter. A similar long-lasting suppression was also observed in vivo by Snider et al. (1967) in the chronically isolated cerebellum. Although they identified the suppression with the basket cell inhibition, the latter does not sustain for more than 0.2 sec after the end of repetitive stimulation (Andersen et al., 1964). In order to explain the suppression lasting for several seconds, we must assume either the presence of inhibitory potential of long duration as observed in sympathetic ganglia (Libet et al., 1968) and invertebrates (Tauc, 1969), or the presence of a neuron circuit continuously activating inhibitory mechanisms for several seconds.

Neurons in the Purkinje cell layer repeated spontaneously a cycle of firing and of silent periods. During the firing period, white matter stimulation induced a pause of firing due to hyperdepolarization originally observed by Granit and Phillips (1956) after fastigial stimulation. They considered this phenomenon as an excessive depolarization of Purkinje cells which inactivated spike generation. In about a half of the cells in the present experiments, a sequence of spike bursts took place at the transition from the firing to silent period. This was similar to the pause of firing due to hyperdepolarization since spikes occurred at high frequencies while rapidly decreasing in height and reversing in polarity at the end of
the burst. A similar change in spike height and polarity was observed in vivo by Brookhart et al. (1950) during application of strychnine, and in these experiments during application of glutamate though reversal in spike polarity was less manifest in the latter case. Whereas spike inactivation was observed in vivo only after high frequency stimulation or during application of strychnine (Brookhart et al. 1950; Granit and Phillips, 1956), it occurred spontaneously in cerebellar sections at the end of the firing period. The fact that spike inactivation is liable to take place in vitro may be explained by the assumption that Golgi cell axons and axon collaterals of Purkinje cells were cut during preparation of the tissue and consequently excitatory processes were dominant over inhibitory ones. In addition, dendritic branches were probably sectioned in a number of neurons which were expected to have reduced membrane potentials and therefore to be readily brought into excessive depolarization.

REFERENCES


Murphy, J. T. and Sabah, N. H. (1970) Spontaneous firing of cerebellar Purkinje cells in


