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EXTRACELLULAR ACTIVATION AND MEMBRANE CONDUCTANCES OF NEURONES IN THE GUINEA-PIG DEEP CEREBELLAR NUCLEI *IN VITRO*

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SUMMARY

1. The responses of cerebellar nuclear cells to extracellular stimulation in a slice preparation were studied and the ionic basis of their electroresponsiveness was investigated with blockers of membrane conductances and with ion substitutions in the extracellular medium.

2. The cells could be activated antidromically from the cerebellar cortex and the white matter surrounding the nuclei. The dominating response to orthodromic stimulation was an inhibitory synaptic potential presumably produced by activation of Purkinje cell fibres.

3. The action potentials and the subthreshold spikelets were shown to be Na^+ dependent and are presumably generated by a voltage-dependent inactivating Na^+ conductance.

4. Plateau potentials with a low threshold were also Na^+ dependent, but these long-lasting potentials are probably produced by activation of a voltage-dependent non-inactivating Na^+ conductance.

5. Plateau potentials with a high threshold and high-threshold spikelets were Ca^{2+} dependent and seem to be generated by non-inactivating and possibly inactivating Ca^{2+} conductances.

6. The spike after-hyperpolarizations had an early voltage-dependent K^+ component and a late Ca^{2+}-dependent K^+ component. They are therefore produced by voltage-sensitive and Ca^{2+}-dependent K^+ conductances.

7. By analogy with the distribution of conductances in Purkinje cells it is proposed that the Na^+ conductances are mainly located in the somatic and axonal membrane and that the Ca^{2+} conductances are located in the dendrites. The functional implications of the complex electroresponsive properties of cerebellar nuclear cells are discussed.

INTRODUCTION

In an accompanying paper (Jahnsen, 1986) the general electrophysiological characteristics of deep cerebellar nuclear neurones (d.c.n. neurones) in a slice preparation were reported. It was found that these cells like their *in vivo* counterparts are spontaneously active and that in addition to the action potential they have three

subthreshold active responses: spikelets, plateau potentials and spike after-hyperpolarizations.

The results were obtained by intracellular stimulation and recording. In this paper the responses of the d.c.n. neurons *in vitro* to extracellular stimuli are presented and the ionic basis of their electroresponsiveness is investigated. In particular it will be shown that the cells can be activated antidromically and synaptically and that activation of corticofugal fibres produces inhibition of the cells. The active responses of the d.c.n. neurones are most likely generated by a number of inactivating and non-inactivating Na^+ and Ca^{2+} conductances as well as voltage- and Ca^{2+}-dependent K^+ conductances. A simple model of the d.c.n. neurones with the distribution of the conductances is presented at the end. Part of this work has been published previously (Jahnsen, 1984 *a*, *b*).

METHODS

The solutions and procedures used for the preparation of the slices and for recording the cells were the same as described in the accompanying paper (Jahnsen, 1986). In a few cases the slices were cut in the parasagittal or horizontal plane instead of the frontal plane in order to preserve different parts of the cerebellar circuitry.

Extracellular stimulation was obtained by placing a pair of 50 μm thick platinum wires spaced approximately 100 μm in the white matter surrounding the cerebellar nuclei or in the cerebellar cortex.

When Co^{2+}, Mn^{2+} or Ba^{2+} was added to the medium $H_2PO_4^-$ was substituted with Cl^- to avoid precipitation. In some experiments with Na^+-free solutions NaCl and $NaHCO_3$ were substituted with choline Cl and choline HCO_3 on an equimolar basis. Tetrodotoxin (TTX; Sigma) was used in a concentration of 1 μg/ml, tetraethylammonium bromide (TEA Br) (Sigma) at 5 mM, 4-aminopyridine (Merck) at 0·5 mM and picrotoxin (Sigma) at 0·1 mM.

During extracellular stimulation the transmembrane potential of the neurones was measured differentially between an intracellular KCH_3SO_4 electrode and an extracellular 0·3 M-NaCl electrode placed as close to the cell as possible. The zero-level was then defined as the potential difference between the two electrodes when they were both extracellular. Although this approach is not flawless it turned out to give a reasonable stability. In a few cases two electrodes were not used during extracellular stimulation. In these cases the recording electrode was withdrawn 5–10 μm from the cell after the experiment and the extracellular stimulus artifact and field potential recorded. The extracellular signal was then subtracted from the intracellular signal off-line in order to get the true transmembrane potential. The results obtained with these two methods were identical.

RESULTS

Responses to extracellular stimulation

Antidromic activation. When the extracellular stimulus electrode was placed in the white matter surrounding the cerebellar nuclei the most common response was an antidromic activation of the d.c.n. neurones. This was true both when the stimulus electrode was near the cerebellar pedunculi or in the white matter between the nuclei and the cerebellar cortex. It suggests that both axons leaving the cerebellum and nucleo-cortical recurrent collaterals are preserved in the preparation. These collaterals have been demonstrated anatomically (Ramon y Cajal, 1911; Hess, 1982) as well as electrophysiologically (Tolbert, Bantli & Bloedel, 1976).

The antidromic spike can be seen in Fig. 1*A*. It was characterized by a rather high

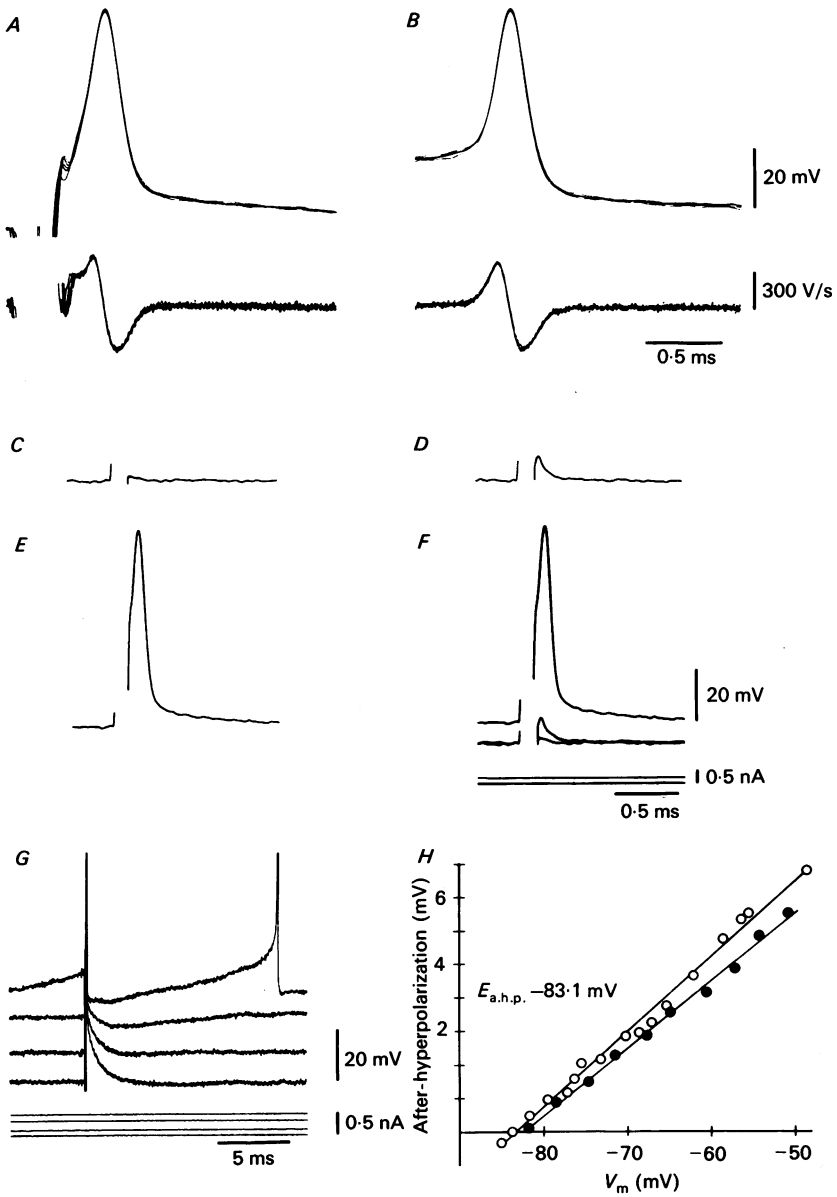

Fig. 1. Antidromic activation of d.c.n. neurones. *A*, antidromic spike (upper trace) and the first derivative dV/dt. Note the notch in the differentiated signal indicating a break in the rising phase of the action potential. *B*, as in *A*, but spontaneous spike without a break. *C*, *D*, *E* and *F*, antidromic stimulation of cell from hyperpolarized level produced no response in *C*. Slight increase of the stimulus intensity (*D*) elicited a small spike. Reduction of the steady hyperpolarizing current and stimulation with the same extracellular pulse generated a full-blown action potential (*E*). The three traces with the holding currents are superimposed in *F*. *G*, reversal of the after-hyperpolarization by hyperpolarization of the membrane with intracellularly injected current. *H*, plots of the amplitude of the after-hyperpolarization measured 10 ms after stimulation as a function of membrane potential (V_m) in two cells. The reversal potential ($E_{a.h.p.}$) was found by linear regression analysis to be −83·1 mV ($r > 0{\cdot}99$ for both regression lines).

rate of rise and with a break occurring during the depolarization. This break is seen as a notch in the differentiated signal (lower trace). For comparison the spontaneous action potential and its first derivative from the same cell are given in Fig. 1*B*. Note that there is no break in the rising phase of the spontaneous potential. In Fig. 1*C*,

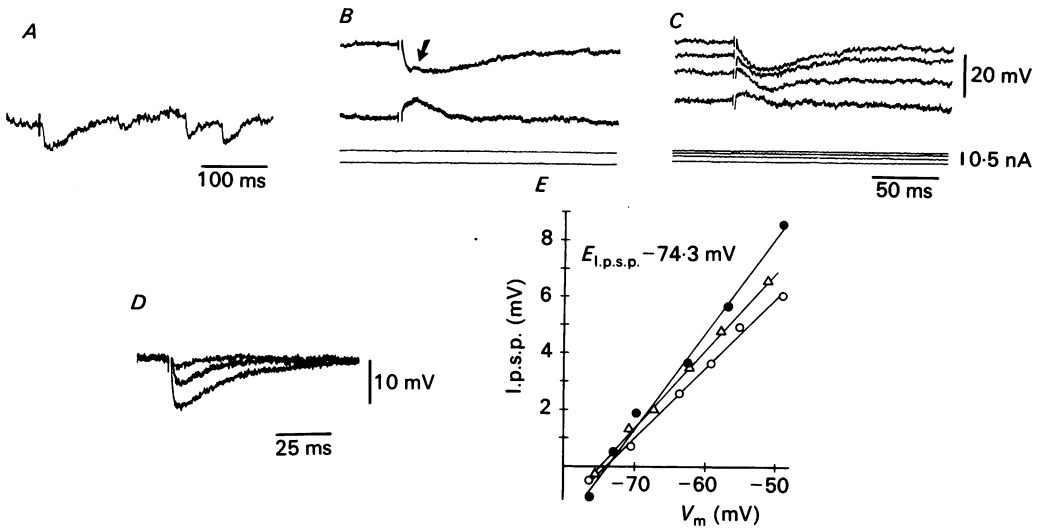

Fig. 2. Synaptic potentials. *A*, inhibitory synaptic potential produced by stimulation of the lateral part of the cerebellar cortex. Spontaneous i.p.s.p.s were also seen in this cell. *B*, stimulation of the perinuclear white matter produced a mixed e.p.s.p.–i.p.s.p. The e.p.s.p. is seen as a little hump (arrow) in the upper trace. As the membrane was hyperpolarized to the reversal potential for the i.p.s.p. the e.p.s.p. became evident. *C*, reversal of a pure i.p.s.p. Note that the early part reverses at more positive membrane potentials. *D*, increasing stimulus intensities generated i.p.s.p.s with increasing amplitudes. *E*, plots of the amplitude of the i.p.s.p. 10 ms after stimulation as a function of the membrane potential (V_m) for three cells. The reversal potential ($E_{i.p.s.p.}$) was found by linear regression analysis to be −73·4 mV ($r > 0{\cdot}98$ for all three regression lines).

D and *E* are shown three records from a neurone stimulated antidromically under different conditions. In Fig. 1*C* the neurone was hyperpolarized with a steady inward current injected through the recording electrode. The stimulus was too small to produce a spike. When the intensity was increased slightly (Fig. 1*D*) the response was a small spike with an amplitude of about 8 mV. By reducing the holding current (Fig. 1*E*) the same stimulus now produced a full-blown action potential. The three sweeps are superimposed in Fig. 1*F*. This experiment shows that hyperpolarization of the neurone can block the invasion of the spike in the d.c.n. cell just as seen in the motoneurone (Coombs, Eccles & Fatt, 1955). In the motoneurone there are usually three components: the M spike produced at the first node of Ranvier, the IS spike from the initial segment and the SD spike from the somato-dendritic membrane. The partially blocked spike in this d.c.n. neurone has the size of an M spike in motoneurones. However, the axon of the d.c.n. neurones can originate on a dendrite up to several hundred micrometres from the soma (Chan-Palay, 1977; see also

Jahnsen, 1986, Pl. 2). Furthermore, a three-step blockage as in motoneurones was never seen in d.c.n. cells *in vitro*: it was either a two-step or an all-or-none event. Probably the partially blocked spike of the cerebellar nuclear cells is equivalent to the IS spike of motoneurones. The size (which never exceeded 15 mV) can be explained by attenuation due to the distance between the recording electrode and the initial segment.

The antidromic spike was followed by an after-hyperpolarization or an after-depolarization depending on the membrane potential. In Fig. 1*G* are shown a series of superimposed sweeps illustrating the voltage dependency of the after-potential. The cell was gradually hyperpolarized with a steady inward current and activated antidromically from different levels. In Fig. 1*H* is plotted the amplitude of the after-potential as a function of the membrane potential. The plot is close to linear and shows that the after-potential reverses at about −83 mV, i.e. close to the equilibrium potential for K^+ (about −84 mV). Thus, this experiment indicates that the spike after-hyperpolarization is produced by a K^+ conductance increase as in other central neurones.

Orthodromic stimulation. It was in general rather difficult to elicit synaptic potentials in the slice preparation. This was the case even though the plane of sectioning was changed in some experiments. Often the synaptic potentials were superimposed on the antidromic response so a detailed analysis was difficult. However, in a few cases inhibitory post-synaptic potentials (i.p.s.p.s) were produced without any contamination and in some cells spontaneous i.p.s.p.s were recorded (Fig. 2*A*).

The i.p.s.p. was generated following stimulation of the cerebellar cortex or the white matter surrounding the cerebellar nuclei with square-wave stimuli up to 500 μA lasting 50–400 μs. It was a hyperpolarizing potential lasting 50–100 ms with an amplitude of 15 mV or less.

The excitatory post-synaptic potential (e.p.s.p.) was never recorded in isolation. There was always an i.p.s.p. or an antidromic response present at the same time (Fig. 2*B*). Therefore a detailed analysis of the e.p.s.p. was not attempted. The e.p.s.p. could be seen as a depolarizing potential with an amplitude up to 10 mV when fibres near the cerebellar peduncles were stimulated and the d.c.n. neurones hyperpolarized with an inward current.

The i.p.s.p. could be reversed by hyperpolarizing the d.c.n. cell membrane (Fig. 2*C*). Note that the early part reverses before the late part. This indicates that the inhibitory synapses are distributed over proximal as well as distal parts of the cell and this is in good agreement with anatomical observations (Chan-Palay, 1977).

The time course and the amplitude of the i.p.s.p. were similar to some of the spike after-hyperpolarizations recorded. However, there were clear differences between the two potentials. The i.p.s.p. was a graded potential and did not necessarily follow an action potential. The reversal potential for the i.p.s.p. (Fig. 2*E*) measured at the time of peak was about 9 mV more positive than that measured for the after-hyperpolarization. Finally, in two cells picrotoxin blocked the i.p.s.p. produced by stimulation of the cortex as it does in Deiter's neurones *in vivo* (Obata, Takeda & Shinozaki, 1970), but the after-hyperpolarization remained unaffected. These

experiments support the hypothesis that the i.p.s.p. seen in d.c.n. cells after cortical stimulation is generated by release of γ-aminobutyric acid (GABA) from Purkinje cell terminals followed by an increased Cl^- conductance of the post-synaptic membrane (ten Bruggencate & Engberg, 1971).

A point of interest in relation to the i.p.s.p. in cerebellar nuclear cells is the interaction with the subthreshold potentials, particularly the plateau potential. The

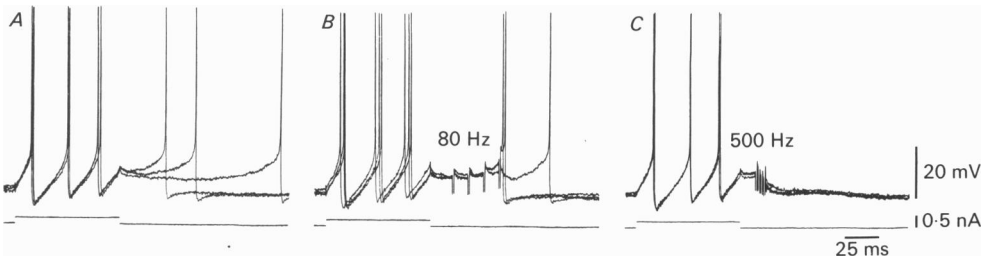

Fig. 3. Interaction between i.p.s.p.s and the plateau potential. *A*, intracellular depolarizing current pulse produced firing. At the break of the current a plateau with spikes was seen. *B*, stimulation of the cerebellar cortex with four pulses at 80 Hz delayed the spiking on the plateau. *C*, the same stimulus, but at 500 Hz terminated the plateau and no spikes were generated.

cell in Fig. 3 was stimulated with an outward current pulse and responded with three action potentials followed by a plateau after the break of the stimulus. The plateau was large enough to produce an additional spike in each trial (Fig. 3*A*). When the cerebellar cortex was stimulated at 80 Hz with four square-wave pulses the spikes on the plateau were delayed, but not abolished and the plateau itself was unaffected (Fig. 3*B*). When the frequency of stimulation in the cortex was increased to 500 Hz (Fig. 3*C*) the plateau potential was terminated and the spikes abolished.

The experiment illustrated in Fig. 3 indicates that low- and high-frequency activation of Purkinje cells may have completely different effects in the cerebellar nuclei. Low-frequency activity may temporarily inhibit a spontaneously active d.c.n. cell or reduce the rate of firing whereas high-frequency activity of the Purkinje cell may convert the d.c.n. neurone from a spontaneously active cell to a silent cell. However, it is not certain that the same number of afferents were stimulated at 80 Hz as at 500 Hz. Therefore no definitive conclusion can be drawn from the experiment. The hypothesis needs further testing in preparations with the complete cerebellar circuitry intact.

Ionic basis of the electroresponsiveness of d.c.n. neurones

In the preceding paper (Jahnsen, 1986) a number of active responses of the d.c.n. neurones were reported. These included an action potential of short duration and below the threshold three slower potentials: spikelets, the plateau and the spike after-hyperpolarization. In order to determine the ionic dependency of these potentials a number of ion substitutions and pharmacological experiments were performed.

The action potential and the spikelets. In all preparations investigated TTX has been

shown to block the fast action potential generated by the inactivating Na^+ conductance (Blankenship, 1976). When TTX was added to the Ringer solution there was a complete block of the spike within a few minutes (Fig. 4*A*, *B*, *C* and *D*). The delay was probably due to the time it took to renew the solution in the recording chamber because when TTX was added directly to the surface of the slice the block was

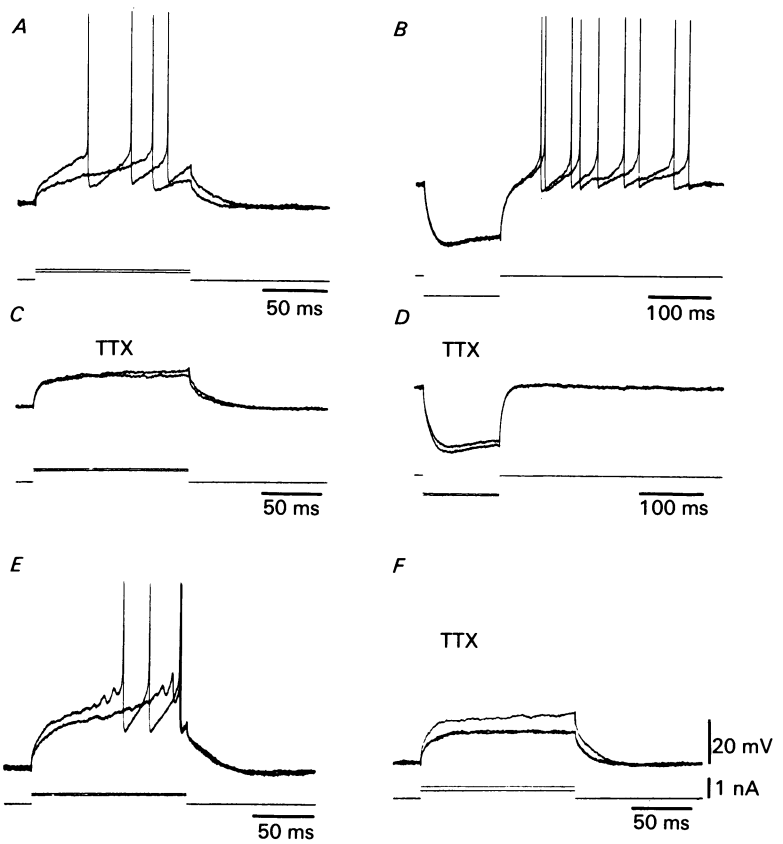

Fig. 4. Effect of TTX on spikes and spikelets. *A* and *B*, control, a depolarizing current pulse produced firing (*A*) and after a hyperpolarizing pulse (*B*) a rebound response was seen. *C* and *D* as *A* and *B*, but after addition of TTX to the medium. All the spikes were blocked. *E*, another cell stimulated with depolarizing current pulses. Spikes and spikelets were generated. *F*, TTX blocked both spikes and spikelets.

complete within seconds. This method was not used in general, however, because of the mechanical instability it caused.

The addition of TTX to the solution also blocked the spikelets (Fig. 4*E* and *F*). In the control solution the cell was stimulated with an outward current pulse producing spikelets and action potentials. After the addition of TTX the action potentials as well as the spikelets were absent even though the stimulus was increased. These experiments support the hypothesis that the action potential is generated by the inactivating Na^+ conductance originally described by Hodgkin & Huxley (1952). Since TTX is considered a specific blocker of a Na^+ conductance it is likely that

this Na^+ conductance also is the basis of the spikelets. That the action potential and the spikelets are Na^+-dependent potentials is further documented by the experiments shown in Figs. 5 and 6.

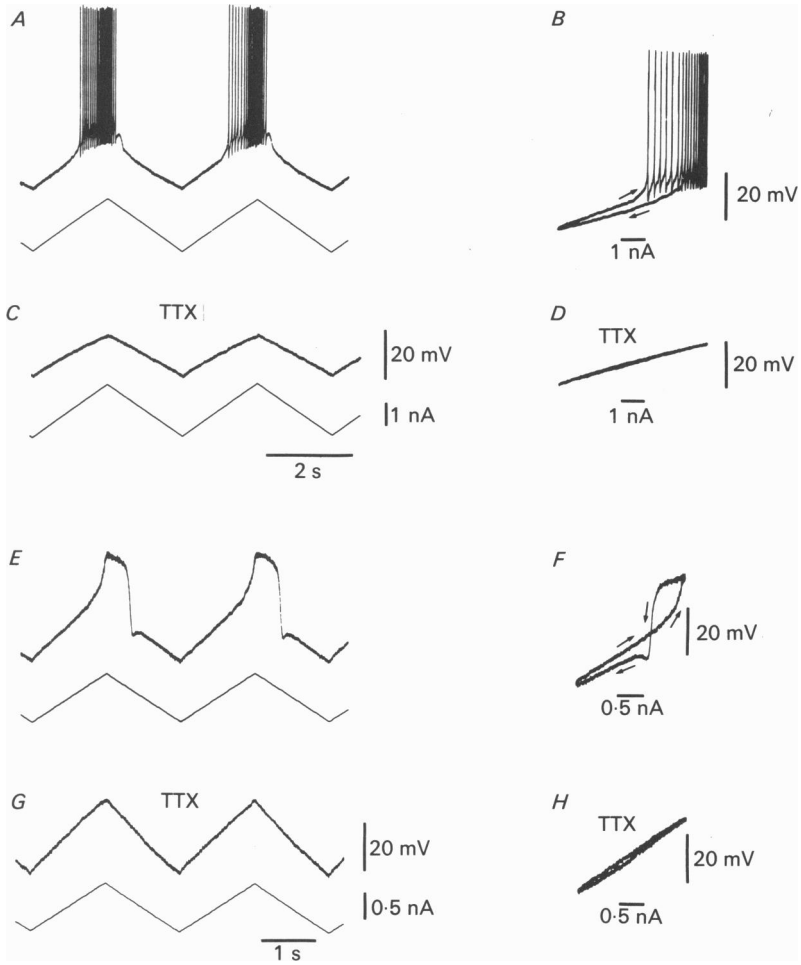

Fig. 5. Effect of TTX on the plateau potentials. *A* and *B*, injection of a double-ramp current (*A*) and the corresponding Lissajous figure (*B*). Note the increasing steepness during the depolarizing phase and the hysteresis in *B*. *C* and *D* as *A* and *B*, but after TTX was added. The response of the membrane became passive as indicated in the straight Lissajous figure without hysteresis. *E* and *F* from another cell with a marked plateau. The action potentials were temporarily inactivated by a preceding strong depolarizing current injection. The plateau potential is seen as the hysteresis in the upper part of the Lissajous figure in (*F*). *G* and *H* after TTX. The plateau was completely blocked.

The plateau potential. Since the plateau potentials are long-lasting in d.c.n. neurones they were particularly well suited for experiments with slow stimuli such as the double-ramp currents shown in Fig. 5. In the upper row a double-ramp current was used to obtain a Lissajous figure as in the preceding paper (Jahnsen, 1986). Note that there was an increasing steepness of the potential as the threshold for the action

potential was approached. After TTX was added to the medium (Fig. 5*C* and *D*) the spikes disappeared, and the increase in steepness of the potential was also blocked. Thus, the process generating the plateau potential is TTX sensitive. In order to study the effect of TTX more clearly another cell with a well-developed plateau was first depolarized to a level where action potentials were blocked with a strong outward

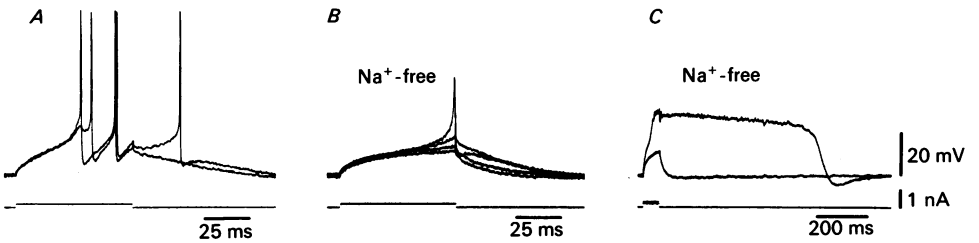

Fig. 6. Effect of substituting Na^+ with choline ions. *A*, control, a depolarizing current pulse produced firing and a plateau response after the break of the current. *B*, as Na^+ was substituted with choline ions the spikes and the plateau were blocked. The responses were obtained as choline entered the recording chamber. The smallest response was generated 7·5 s after the largest. *C*, 5 min after substitution with choline. The small response was produced with a stimulus identical to those used in *A* and *B*. When the amplitude of the stimulus was increased a new plateau potential was generated.

current for about 1 min. When the current was turned off the action potential remained inactivated for some time, but the plateau was unaffected (Fig. 5*E*). The Lissajous figure from this cell (Fig. 5*F*) clearly showed the plateau as a marked hysteresis in the upper half of the plot, but note also that there was some hysteresis in the lower half. This is probably due to activation of K^+ current by the preceding depolarization (see below). TTX completely blocked the plateau (Fig. 5*G* and *H*). The membrane was passive in the response to the double-ramp current injection and the hysteresis of the Lissajous figure disappeared. These experiments indicate that at least some plateau potentials and the anomalous rectification in the depolarizing direction are generated by a non-inactivating Na^+ conductance as originally observed in the Purkinje cell (Llinás & Sugimori, 1980*a*).

In addition to using TTX the Na^+ dependency of the electroresponsiveness of d.c.n. neurones was studied by substituting Na^+ with the impermeant choline ion. Fig. 6*A* shows a cell having both spikelets and action potentials in response to a depolarizing current injection. After the current pulse a small plateau with spikes was seen. When the recording chamber was perfused with a choline-containing solution all of the above responses were blocked within seconds. Fig. 6*B* shows four successive responses from the cell at the time choline entered the chamber. The interval between each stimulus was 2·5 s and the block was thus complete within 10 s. However, this experiment demonstrated that not all plateau potentials were generated by Na^+-dependent processes. When the stimulus was increased another plateau potential with a high threshold was produced (Fig. 6*C*). This was not only seen in cells exposed to choline ions. Cells treated with TTX also sometimes had plateau potentials with a high threshold. In fact, the experiment in Fig. 7 illustrates that cells poisoned with TTX could always generate a plateau potential with a high threshold provided some

of the voltage-dependent K^+ conductance of the membrane was blocked with TEA (Armstrong & Binstock, 1965). In Fig. 7*A* the cell was stimulated with a short current pulse producing a spike, spikelets and a long plateau. After the addition of TTX the membrane apparently became passive, but as the stimulus was increased it could be seen that the membrane potential did not follow the smooth exponential trajectory expected from a passive response (Fig. 7*B*). Then the K^+ conductance of the

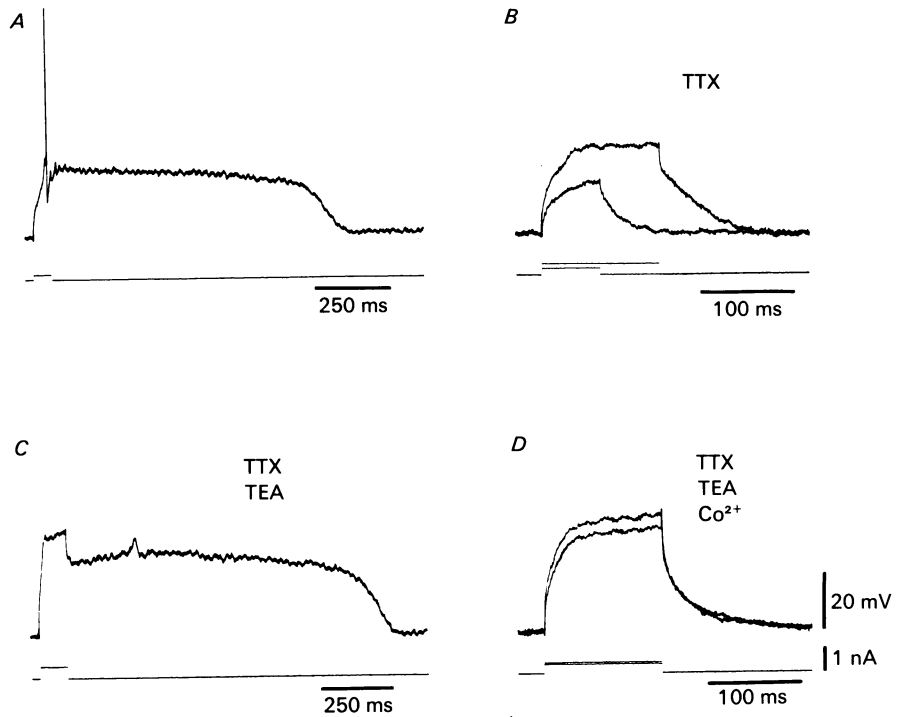

Fig. 7. Ionic basis of plateau potentials with a low and a high threshold. *A*, control, a depolarizing current pulse produced a spike, spikelets and a plateau potential. *B*, TTX blocked the spike, the spikelets and the plateau, but the relaxation of the membrane potential at the break of the larger current pulse was not passive. Instead of an exponential decay the potential fell at a steady rate. Note also the break during the rising phase of this response. *C*, addition of TEA to the medium. A new plateau potential with a higher threshold could be generated. Note also the long-lasting spikelet on the plateau. *D*, addition of Co^{2+} blocked the plateau and the spikelet. The response was probably passive, but rectification in the recording electrode caused an increase of the potential during the late part of the current pulse.

membrane was reduced by adding TEA to the solution (Fig. 7*C*), and a new plateau with a high threshold was elicited with the current pulse. Note also that a new type of spikelet with a duration of about 30 ms and an amplitude of about 6 mV was generated on top of the plateau. Finally, Ca^{2+} was substituted with Co^{2+} in the perfusion medium (Fig. 7*D*) and this blocked the plateau as well as the high-threshold spikelet. In conclusion, then, the d.c.n. neurones have Na^+-dependent plateau potentials and spikelets below the threshold for the action potential. When the Na^+

conductance of the membrane is blocked the cells can still generate plateau potentials and spikelets provided that some of the K^+ conductance of the membrane is blocked. In fact, in a few cells it was not necessary to block the K^+ conductance to obtain the high-threshold potentials. The high-threshold plateau and spikelets are Ca^{2+}-dependent potentials because they were blocked when Ca^{2+} was substituted with Co^{2+} (Hagiwara, 1973).

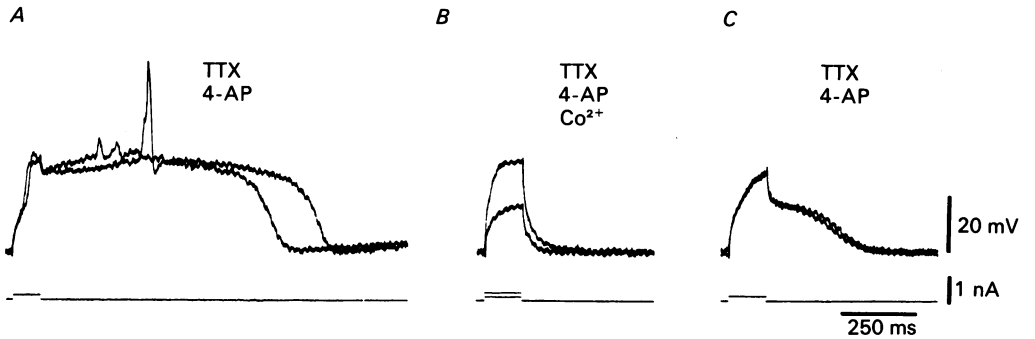

Fig. 8. Effect of 4-AP and Co^{2+} on the high-threshold spikelets and plateau. *A*, in the presence of TTX and 4-AP a high-threshold plateau response was produced by injection of an outward current pulse. A TTX-resistant spike and several spikelets were riding on the plateau. *B*, addition of Co^{2+} blocked the plateau and the spikelets. *C*, partial recovery after Co^{2+} was removed from the medium.

It has been shown that 4-aminopyridine (4-AP) potentiates dendritic Ca^{2+} spikes in hippocampal CA3 cells (Gustafsson, Galvan, Grafe & Wigström, 1982) and thalamic neurones (Jahnsen & Llinás, 1984) presumably by blocking K^+ conductance. The addition of 4-AP to the solution also increased the number and amplitude of the TTX-resistant high-threshold spikes in d.c.n. neurones (Fig. 8*A*) and substitution of Ca^{2+} with Co^{2+} was an effective blocker of the plateau and the spikes in this situation as well (Fig. 8*B*). Similar results were obtained when Mn^{2+} was used instead of Co^{2+}. When the cell was washed in a Ca^{2+}-containing solution the plateau reappeared in part, but the spikes never came back. This may indicate that the high-threshold plateau and the high-threshold spikes are generated by separate Ca^{2+}-dependent processes with different sensitivities to Ca^{2+} conductance blockers.

The spike after-hyperpolarization. It was shown in Fig. 1 that the after-hyperpolarization reversed at a potential close to the equilibrium potential for K^+. The ionic dependency of this response was investigated by three types of experiments. A d.c.n. cell was stimulated with a depolarizing current pulse near the threshold and responded with fast spikes followed by marked after-hyperpolarizations (Fig. 9*A*). When the Ca^{2+} of the perfusion medium was substituted with Mn^{2+} as in Fig. 9*B* or Co^{2+} in other experiments the after-hyperpolarizations became greatly reduced in amplitude as well as in duration. The difference is seen more clearly in Fig. 9*C* where responses before and after substitution with Mn^{2+} are superimposed. This experiment suggests that the spike after-hyperpolarization is generated by a Ca^{2+}-dependent process, presumably the Ca^{2+}-dependent K^+ conductance known from other central

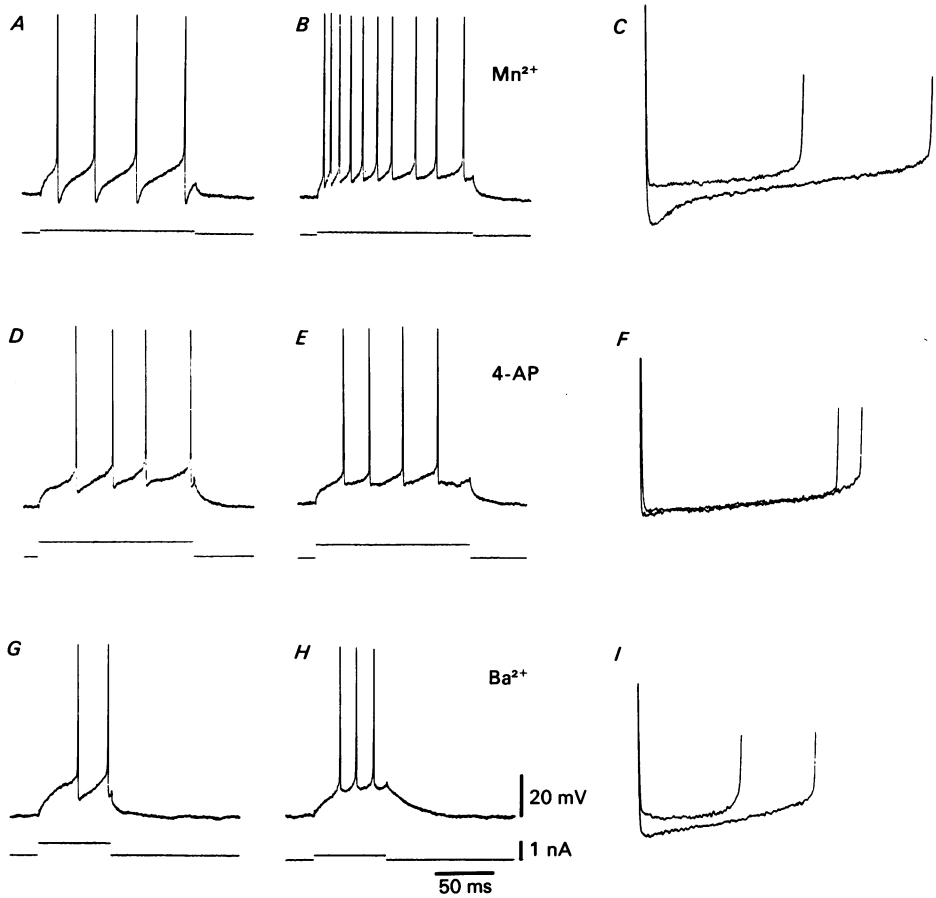

Fig. 9. Ionic basis of the after-hyperpolarization. *A*, *B* and *C*, effect of Mn^{2+}. A depolarizing current pulse was injected and a short train of spikes followed by after-hyperpolarizations was generated (*A*). When Mn^{2+} was added the after-hyperpolarizations were reduced and the firing frequency went up (*B*). Interspike trajectories from *A* and *B* are superimposed in *C*. The upper trace was obtained in the presence of Mn^{2+}. *D*, *E* and *F*, as in *A*, *B* and *C*, but with addition of 4-AP instead of Mn^{2+}. Note that the effect of 4-AP was very small (*F*), the spike lasted slightly longer and the earliest part of the after-hyperpolarization was reduced. *G*, *H* and *I* as *A*, *B* and *C*, but with addition of Ba^{2+}. Note that a hyperpolarizing holding current had to be injected when Ba^{2+} was added to maintain the membrane potential and that the input resistance of the membrane went up (*H*). Ba^{2+} reduced the after-hyperpolarization as Mn^{2+} (*I*).

neurones (Hotson & Prince, 1980; Llinás & Sugimori, 1980*a*, *b*; Llinás & Yarom, 1981; Jahnsen & Llinás, 1984; Llinás, Greenfield & Jahnsen, 1984).

In order to study the possible contributions of voltage-dependent K^+ conductances to the after-hyperpolarizations TEA was initially tried. However, the addition of this drug led to the generation of long plateau potentials as mentioned earlier, masking the effect on the spike after-hyperpolarizations. 4-AP, however, has also been reported to block some voltage-dependent K^+ currents in mammalian C.N.S. cells, especially the A current (Yarom, Sugimori & Llinás, 1980; Gustafsson *et al.* 1982)

and the delayed rectifier (Galvan, Grafe & ten Bruggencate, 1982) whereas the effect on the Ca^{2+}-dependent K^+ current is insignificant (Rogawski & Barker, 1983). Addition of 4-AP to the bathing solution did not affect the later part of the after-hyperpolarizations (Fig. 9*D* and *E*), but note that when the responses are superimposed as in Fig. 9*F* it can be seen that the spike lasted slightly longer and that the early after-hyperpolarization was reduced by 4-AP. The after-hyperpolarizations may therefore consist of an early part generated by a voltage-dependent K^+ conductance lasting a few milliseconds and a late part produced by a K^+ conductance increase activated by Ca^{2+} entry. The small after-depolarization may occur in cells where the voltage-dependent K^+ conductance inactivates before the Ca^{2+}-dependent K^+ conductance is fully activated.

Finally Ba^{2+} was used to investigate the after-hyperpolarizations. Ba^{2+} is known to move through Ca^{2+} channels (Werman & Grundfest, 1961; Hagiwara, 1973), but the Ca^{2+}-dependent K^+ conductance is not activated by Ba^{2+} (Eckert & Lux, 1976). Substitution of Ca^{2+} with Ba^{2+} had a marked effect (Fig. 9*G*, *H* and *I*). The after-hyperpolarizations were greatly reduced, but note also that the input resistance of the membrane went up and that it was necessary to increase the inward holding current to maintain the membrane potential. Thus, Ba^{2+} not only blocked the after-hyperpolarizations, it also decreased the net outward current of the membrane at subthreshold potentials. Ba^{2+} is known to block the M current carried by K^+ and produce depolarization in hippocampal neurones (Halliwell & Adams, 1982). It is thus possible that the Ba^{2+}-induced depolarization seen here was produced by a reduction of a similar current. In conclusion, then, the spike after-hyperpolarization is probably generated by a Ca^{2+}-dependent K^+ current, but voltage-dependent processes such as the delayed rectifier blocked by TEA and other K^+ currents probably also influence the membrane potential between spikes.

DISCUSSION

This paper has dealt with the responses of d.c.n. neurones to extracellular stimuli and with the ionic requirements of their electroresponsive properties.

It has been found that the cells can be activated antidromically from the white matter surrounding the nuclei as well as from the cerebellar cortex. The shape of the antidromic spike suggests that it consists of an axonal component and a somato-dendritic component. The dominating orthodromic response to cortical stimulation is an i.p.s.p. with the same characteristics as the one seen in lateral vestibular neurones *in vivo*, i.e. a hyperpolarizing graded potential probably produced by a Cl^- conductance increase activated by GABA release from the presynaptic terminals of the Purkinje cells (Obata *et al.* 1970; ten Bruggencate & Engberg, 1971). It has also been demonstrated that high-frequency activation of inhibitory afferents to the d.c.n. neurones is more effective in producing inhibition than low-frequency activation.

The electroresponsiveness of the d.c.n. cells was investigated with ion substitutions in the extracellular medium and with drugs blocking various conductances of nerve membranes. The action potential and the low-threshold spikelets and plateau potentials are Na^+ dependent and the high-threshold spikes and plateau potentials are Ca^{2+} dependent. Furthermore, the spike after-hyperpolarization is mainly

produced by a Ca^{2+} dependent process with a reversal potential close to the equilibrium potential for K^+; i.e. most likely a Ca^{2+} dependent K^+ conductance.

The responses to extracellular stimulation

The antidromic activation of the d.c.n. neurones produced spikes with a break in the rising phase as seen in motoneurones (Coombs *et al.* 1955) and in d.c.n. neurones *in vivo* (Tolbert *et al.* 1976). Hyperpolarization of the membrane prior to the antidromic stimulus could sometimes reduce the amplitude of the action potential to 5–15 mV. This may be an equivalent of the IS spike of motoneurones. In cells where a break in the action potential occurred above one-third of the full depolarization it was never possible to isolate an IS spike by hyperpolarization. This may indicate that in cases where the initial segment is close to the soma the IS spike is always capable of firing the somato-dendritic membrane because of the relatively high input resistance of the soma and the dendrites. This is in contrast to the situation in motoneurones where the input resistance of the soma and the dendrites is low enough to prevent the membrane potential of the somato-dendritic membrane from reaching threshold. In cases where the axon of the d.c.n. neurones takes off from a dendritic branch the IS spike can be isolated presumably because the dendrite has a high threshold for spike generation and because the IS spike is attenuated by a high internal resistance of the dendrite and possibly by a voltage-sensitive resistance decrease of the dendritic membrane. This is also the reason why the IS spike is smaller in d.c.n. neurones than in motoneurones. It should be mentioned, however, that in some of the deteriorating d.c.n. neurones not included among the sixty cells of this report a block of the SD spike occurred leaving an IS spike with an amplitude of 35–40 mV.

There is an important difference between the antidromic spike and the spontaneously generated spike in that the first has a break and the latter a smooth trajectory. However, the duration of the two spikes is the same. This leads to the suggestion that the spontaneous spike is produced by a depolarizing process located in the somato-dendritic membrane. This process is continuously active in d.c.n. neurones and is most likely identical to the one generating plateau potentials in these cells, i.e. a non-activating inward current with a low threshold (see below).

The synaptic activation of the d.c.n. neurones *in vitro* was substantially more difficult than in other *in vitro* preparations as for instance the hippocampal slice. This is probably due to cutting of the afferent fibres during the sectioning. For this reason a proper analysis of the e.p.s.p. was not possible in this study. The i.p.s.p. had properties similar to that recorded *in vivo* (Ito, Yoshida, Obata, Kawai & Udo, 1970). The i.p.s.p. in d.c.n. cells produced after stimulation of the cerebellar cortex lasts about 50–100 ms and has the same duration as the one recorded in cells in the lateral vestibular nucleus (Ito & Yoshida, 1966). The large cells of the lateral vestibular nucleus have a much lower input resistance than the d.c.n. neurones. Therefore, the duration of the i.p.s.p. in the two cell types is probably determined by the transmitter and not by the passive properties of the cell membranes.

There has recently been a discussion of the effect of destruction of the inferior olive on the inhibition produced by Purkinje cells on their target neurones (Ito, Orlov & Shimoyama, 1978; Ito, Nisimaru & Shibuki, 1979; Montarolo, Raschi & Strata, 1981).

It cannot be ruled out that the difficulties in obtaining synaptic potentials in the slice preparation of the cerebellar nuclei could be caused by absence of some sort of 'trophic' factor present when the olivo-cerebellar system is intact.

It has been shown in the cat *in vivo* (Campbell, Ekerot, Hesslow & Oscarsson, 1983; Campbell, Ekerot & Hesslow, 1983) that climbing fibre activation generates plateau potentials in Purkinje cells whereas parallel fibre activation can terminate plateau potentials presumably via disynaptic inhibition. It was not possible to elicit plateau potentials by orthodromic activation in the d.c.n. neurones, but it is very likely that an e.p.s.p. under physiological conditions can set off a plateau potential and thus generate prolonged activity of a nuclear cell. The termination of a plateau potential by i.p.s.p.s shown in this study corresponds to the postulated effect of parallel fibre stimulation on Purkinje cells. Furthermore, it was demonstrated here that the frequency of activity in the inhibitory pathway is important for the effect on the plateau. A frequency similar to the average frequency of simple spikes in Purkinje cells may be insufficient to terminate a plateau, whereas high-frequency activity as seen during complex spikes (Eccles, Llinás & Sasaki, 1966*a*, *b*) is highly effective. The influence of the Purkinje cell i.p.s.p. on the plateau potentials of d.c.n. neurones adds to the growing evidence of interaction between synaptic transmission and the complex electroresponsive membrane properties of mammalian central neurones (cf. Llinás & Sugimori, 1982; Hounsgaard, Hultborn, Jespersen & Kiehn, 1984).

The ionic basis of the d.c.n. cell electroresponsiveness

The ionic dependencies of the responses of the d.c.n. neurones have counterparts in other cells in the mammalian central nervous system. The spike-generating Na^+ conductance and the associated voltage-dependent K^+ conductance (Hodgkin & Huxley, 1952) seem to be ubiquitous. The spikelets were Na^+ dependent and may be generated by the same inactivating Na^+ conductance as a local response. This was suggested for the so-called fast pre-potentials of the hippocampal pyramidal cells (Spencer & Kandel, 1961). The pre-potentials in the hippocampus may also be coupling potentials between neurones (MacVicar & Dudek, 1981), but there is no evidence for electrical coupling in the cerebellar nuclei. The spikelets seem to serve as triggers for the action potential. They may be an important link between e.p.s.p.s and the spike generation. Blockage of the action potential with TTX or by removal of Na^+ revealed that d.c.n. neurones also have high-threshold spikelets. These spikelets are Ca^{2+} dependent and thus similar to high-threshold Ca^{2+} spikes recorded in hippocampal pyramidal cells (Schwartzkroin & Slawsky, 1977), inferior olivary cells (Llinás & Yarom, 1981), dorsal horn neurones (Murase & Randić, 1983), thalamic neurones (Jahnsen & Llinás, 1984) and nigral neurones (Llinás *et al.* 1984).

The low-threshold plateau potentials were generated by a Na^+-dependent process. This is probably a non-inactivating Na^+ conductance known from Purkinje cells (Llinás & Sugimori, 1980*a*), neocortical cells (Stafstrom, Schwindt & Crill, 1982) and thalamic neurones (Jahnsen & Llinás, 1984). The presence of this conductance is probably the main reason for the spontaneous activity of the cells. It is interesting that not all the d.c.n. cells had well-developed plateau potentials. Two reasons may account for this. First, small differences in the quality of the impalement may affect

the plateau which is very sensitive to even minute leak currents. Secondly, it is possible that the density of non-inactivating Na^+ channels in the membrane is a parameter regulated by internal or external factors. Thus, the d.c.n. cells may be able to change their input–output relations by changing the number of plateau-generating channels in their membranes or, alternatively, external factors such as transmitters may determine the electroresponsiveness of the cells by changing the available pool of channels.

The high-threshold plateau potentials were produced by a Ca^{2+} dependent mechanism. It is most likely equivalent to the non-inactivating Ca^{2+} conductance known from motoneurones (Schwindt & Crill, 1977), Purkinje cells (Llinás & Sugimori, 1980*b*), hippocampal cells (Johnston, Hablitz & Wilson, 1980; Brown & Griffith, 1983) and locus coeruleus neurones (Williams, North, Shefner, Nishi & Egan, 1984). In some d.c.n. neurones *in vitro* this conductance produces depolarizations large enough to inactivate the action potential. It is impossible to say whether this occurs under physiological conditions. If this were the case the hyperpolarizing synaptic potential generated by Purkinje cells would have an interesting role. It would be a synaptic potential that will bring the membrane potential back to a level where firing can be resumed, i.e. instead of inhibition the Purkinje cells would produce excitation of the d.c.n. neurones. The function of the non-inactivating Ca^{2+} current may also be to 'boost' e.p.s.p.s generated in the peripheral dendrites.

The Ca^{2+} dependent spike after-hyperpolarization seems to be as omnipresent in mammalian central neurones as the inactivating Na^+ conductance. It is generated by a Ca^{2+}-activated K^+ conductance originally described in invertebrates (see Meech (1978) for a review). The fact that it is activated after a single action potential suggests that Ca^{2+} enters the cell during the spike even though the latter is very fast in d.c.n. neurones. Spikes *per se* are not essential, though, as seen in the Lissajous plot in Fig. 5*F*. Depolarization alone can presumably cause Ca^{2+} entry in the cell and thus activate the K^+ conductance.

The experiments with Ba^{2+} suggest that there is a voltage-dependent K^+ current with properties similar to the M current in mammalian pyramidal neurones (Halliwell & Adams, 1982). The presence of a sag in the potential during hyperpolarizing current pulses indicates that at least some of the d.c.n. neurones have the Q current also described by Halliwell & Adams (1982). It has not been possible to characterize these and other K^+ currents in this report. However, K^+ conductances are important determinants of the electrical properties of neurones so this issue deserves further study.

The types of conductances suggested to be present in the membranes of d.c.n. cells correspond to the conductances described in Purkinje cells (Llinás & Sugimori, 1980*a*, *b*). This may not be a coincidence since the two cell types are closely related ontogenetically. In rats d.c.n. neurones and the Purkinje cells originate from adjacent layers in the cerebellar primordium around gestational day 14 and 15 respectively (Altman & Bayer, 1978; Altman & Bayer, 1985). Furthermore, it is known that the electrical membrane properties of cerebellar neurones are determined at least before gestational day 16 (Hounsgaard & Yarom, 1985). It is therefore possible that the types of channels as well as their distribution may be identical in the two cell types. Because it was possible to identify the position of the recording electrode visually Llinás &

Sugimori (1980*a*, *b*) could determine the location of the conductances in Purkinje cells. By analogy with the Purkinje cells the model shown in Fig. 10 for cerebellar nuclear cells is proposed. Here the Ca^{2+} conductances are located in the dendrites and the Na^+ conductances mainly in the soma and the axon. This model can account for the fact that the Ca^{2+}-dependent potentials had a high threshold to intracellular

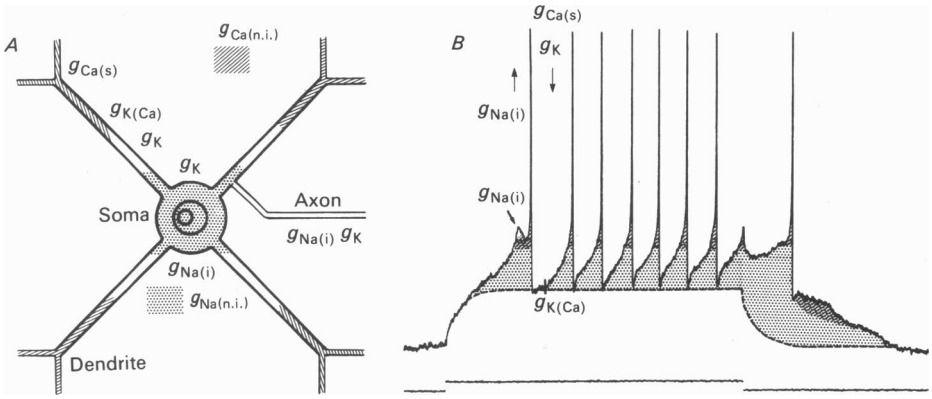

Fig. 10. A model of some conductances and their distribution in d.c.n. neurones. *A*, schematic drawing of a d.c.n. cell. The axon and the soma have an inactivating Na^+ conductance $g_{Na(i)}$ and a voltage-dependent K^+ conductance (g_K) generating action potentials. Patches of membrane in the proximal dendrites also contain $g_{Na(i)}$ capable of generating spikelets with a low threshold. The soma and the proximal dendrites have a non-inactivating Na^+ conductance $g_{Na(n.i.)}$ (dotted area) generating the low-threshold plateau potentials. The distal dendrites have a non-inactivating Ca^{2+} conductance $g_{Ca(n.i.)}$ (hatched areas) producing the high-threshold plateau potentials and possibly a Ca^{2+} spike generating Ca^{2+} conductance $g_{Ca(s)}$. The Ca^{2+}-dependent K^+ conductance $g_{K(Ca)}$ is mainly located in the dendrites. *B*, response of a d.c.n. neurone to a depolarizing current pulse with indications of the contribution of the various conductances to the potential changes. The dashed line shows the expected response of a passive membrane. The dotted area is the contribution of $g_{Na(n.i.)}$ and the hatched area the contribution of $g_{Ca(n.i.)}$. See text for further explanation.

stimulation because they had to be activated through the relatively thin and long dendrites of the d.c.n. cells. The fact that Purkinje cells and d.c.n. cells do not have identical electroresponsive properties can be explained by the difference in morphology. Purkinje cells have well-developed dendrites as compared to d.c.n. cells and thus the Ca^{2+} spikes and Ca^{2+} plateau potentials must be much more prominent in Purkinje cells. This is in good agreement with the data. Other factors such as differences in density of the ion channels in the membranes probably also play a role.

The conclusion of this and the preceding paper (Jahnsen, 1986) is that cerebellar nuclear cells like other central neurones have complex electroresponsive properties. Previous studies of these cells should be seen in the light of that. For instance, it has been postulated that a reverberating neural network exists between the cerebellar nuclei and pontine nuclei (Tsukahara, 1972; Tsukahara, Bando, Murakami & Oda, 1983). It is entirely possible that the observed prolonged depolarizations observed

in pontine neurones after stimulation of d.c.n. neurones or their afferents are produced by prolonged activity of the d.c.n. cells generated by intrinsic plateau potentials and not by reverberating excitatory activity as originally postulated. These and similar questions must be addressed in future studies.

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REFERENCES

ALTMAN, J. & BAYER, A. B. (1978). Prenatal development of the cerebellar system in the rat. I. Cytogenesis and histogenesis of the deep nuclei and the cortex of the cerebellum. *Journal of Comparative Neurology* **179**, 23–48.

ALTMAN, J. & BAYER, S. A. (1985). Embryonic development of the rat cerebellum. I. Delineation of the cerebellar primordium and early cell movements. *Journal of Comparative Neurology* **231**, 1–26.

ARMSTRONG, C. M. & BINSTOCK, L. (1965). Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *Journal of General Physiology* **48**, 859–872.

BLANKENSHIP, J. E. (1976). Tetrodotoxin: from poison to powerful tool. *Perspectives in Biology and Medicine* **19**, 509–526.

BROWN, D. A. & GRIFFITH, W. H. (1983). Persistent slow inward calcium current in voltage-clamped hippocampal neurones of the guinea-pig. *Journal of Physiology* **337**, 303–320.

CAMPBELL, N. C., EKEROT, C.-F. & HESSLOW, G. (1983). Interaction between responses in Purkinje cells evoked by climbing fibre impulses and parallel fibre volleys in the cat. *Journal of Physiology* **340**, 225–238.

CAMPBELL, N. C., EKEROT, C.-F., HESSLOW, G. & OSCARSSON, O. (1983). Dendritic plateau potentials evoked in Purkinje cells by parallel fibre volleys in the cat. *Journal of Physiology* **340**, 209–223.

CHAN-PALAY, V. (1977). *Cerebellar Dentate Nucleus*. Berlin, Heidelberg, New York: Springer-Verlag.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955). The electrical properties of the motoneurone membrane. *Journal of Physiology* **130**, 291–325.

ECCLES, J. C., LLINÁS, R. & SASAKI, K. (1966*a*). The excitatory synaptic action of climbing fibres on the Purkinje cells of the cerebellum. *Journal of Physiology* **182**, 268–296.

ECCLES, J. C., LLINÁS, R. & SASAKI, K. (1966*b*). The action of antidromic impulses on the cerebellar Purkinje cells. *Journal of Physiology* **182**, 316–345.

ECKERT, R. & LUX, H. D. (1976). A voltage-sensitive persistent calcium conductance in neuronal somata of *Helix*. *Journal of Physiology* **254**, 129–151.

GALVAN, M., GRAFE, P. & TEN BRUGGENCATE, G. (1982). Convulsant actions of 4-aminopyridine on the guinea-pig olfactory cortex slice. *Brain Research* **241**, 75–86.

GUSTAFSSON, B., GALVAN, M., GRAFE, P. & WIGSTRÖM, H. (1982). A transient outward current in a mammalian central neurone blocked by 4-aminopyridine. *Nature* **299**, 252–254.

HAGIWARA, S. (1973). Ca spikes. *Advances in Biophysics* **4**, 71–102.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research* **250**, 71–92.

HESS, D. T. (1982). Cerebellar nucleo-cortical neurons projecting to the vermis of lobule VII in the rat. *Brain Research* **248**, 361–366.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *Journal of Physiology* **117**, 500–544.

HOTSON, J. R. & PRINCE, D. A. (1980). A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. *Journal of Neurophysiology* **43**, 409–419.

HOUNSGAARD, J., HULTBORN, H., JESPERSEN, B. & KIEHN, O. (1984). Intrinsic membrane properties causing a bistable behaviour of α-motoneurones. *Experimental Brain Research* **55**, 391–394.

HOUNSGAARD, J. & YAROM, Y. (1985). Intrinsic control of electroresponsive properties of transplanted mammalian brain neurons. *Brain Research* **335**, 372–376.

ITO, M., NISIMARU, N. & SHIBUKI, K. (1979). Destruction of inferior olive induces rapid depression in synaptic action of cerebellar Purkinje cells. *Nature* **277**, 568–569.

ITO, M., ORLOV, I. & SHIMOYAMA, I. (1978). Reduction of the cerebellar stimulus effect on rat Deiters neurons after chemical destruction of the inferior olive. *Experimental Brain Research* **33**, 143–145.

ITO, M. & YOSHIDA, M. (1966). The origin of cerebellar-induced inhibition of Deiters neurones. I. Monosynaptic initiation of the inhibitory postsynaptic potentials. *Experimental Brain Reserarch* **2**, 330–349.

ITO, M., YOSHIDA, M., OBATA, K., KAWAI, N. & UDO, M. (1970). Inhibitory control of intracerebellar nuclei by the Purkinje cell axons. *Experimental Brain Research* **10**, 64–80.

JAHNSEN, H. (1984*a*). Functional differences between neurons in different brain regions. *Acta neurologica scandinavica* **70**, 211*P*.

JAHNSEN, H. (1984*b*). Properties of neurons in the deep cerebellar nuclei recorded *in vitro*. *Acta physiologica scandinavica* **121**, 36*P*.

JAHNSEN, H. (1986). Electrophysiological characteristics of neurones in the guinea-pig deep cerebellar nuclei *in vitro*. *Journal of Physiology* **372**, 129–147.

JAHNSEN, H. & LLINÁS, R. (1984). Ionic basis for the electroresponsiveness and oscillatory properties of guinea-pig thalamic neurones *in vitro*. *Journal of Physiology* **349**, 227–247.

JOHNSTON, D., HABLITZ, J. H. & WILSON, W. A. (1980). Voltage clamp discloses slow inward current in hippocampal burst-firing neurones. *Nature* **286**, 391–393.

LLINÁS, R., GREENFIELD, S. A. & JAHNSEN, H. (1984). Electrophysiology of pars compacta cells in the in vitro substantia nigra – a possible mechanism for dendritic release. *Brain Research* **294**, 127–132.

LLINÁS, R. & SUGIMORI, M. (1980*a*). Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *Journal of Physiology* **305**, 171–195.

LLINÁS, R. & SUGIMORI, M. (1980*b*). Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *Journal of Physiology* **305**, 197–213.

LLINÁS, R. & SUGIMORI, M. (1982). Functional significance of the climbing fiber input to Purkinje cells: an *in vitro* study in mammalian cerebellar slices. In *The Cerebellum–New Vistas*, ed. PALAY, S. L. & CHAN-PALAY, V., pp. 403–409. Berlin, Heidelberg, New York: Springer-Verlag.

LLINÁS, R. & YAROM, Y. (1981). Electrophysiology of mammalian inferior olivary neurones *in vitro*, different types of voltage-dependent ionic conductances. *Journal of Physiology* **315**, 549–567.

MACVICAR, B. A. & DUDEK, F. E. (1981). Electrotonic coupling between pyramidal cells: a direct demonstration in rat hippocampal slices. *Science* **213**, 782–785.

MEECH, R. W. (1978). Calcium-dependent potassium activation in nervous tissues. *Annual Review of Biophysics and Bioengineering* **7**, 1–18.

MONTAROLO, P. G., RASCHI, F. & STRATA, P. (1981). Are the climbing fibres essential for the Purkinje cell inhibitory action? *Experimental Brain Research* **42**, 215–218.

MURASE, K. & RANDIĆ, M. (1983). Electrophysiological properties of rat spinal dorsal horn neurones *in vitro*: calcium-dependent action potentials. *Journal of Physiology* **334**, 141–154.

OBATA, K., TAKEDA, K. & SHINOZAKI, H. (1970). Further study on pharmacological properties of the cerebellar-induced inhibition of Deiters neurones. *Experimental Brain Research* **11**, 327–342.

RAMON Y CAJAL, S. (1911). *Histologie due système nerveux de l'homme et des vertébres*. Paris: Maloine.

ROGAWSKI, M. A. & BARKER, J. L. (1983). Effects of 4-aminopyridine on calcium action potentials and calcium current under voltage clamp in spinal neurons. *Brain Research* **280**, 180–185.

SCHWARTZKROIN, P. A. & SLAWSKY, M. (1977). Probable calcium spikes in hippocampal neurons. *Brain Research* **135**, 157–161.

SCHWINDT, P. & CRILL, W. E. (1977). A persistent negative resistance in cat lumbar motoneurons. *Brain Research* **120**, 173–178.

SPENCER, W. A. & KANDEL, E. R. (1961). Electrophysiology of hippocampal neurons. IV. Fast prepotentials. *Journal of Neurophysiology* **24**, 272–285.

STAFSTROM, C. E., SCHWINDT, P. C. & CRILL, W. E. (1982). Negative slope conductance due to a persistent subthreshold sodium current in cat neocortical neurons *in vitro*. *Brain Research* **236**, 221–226.

TEN BRUGGENCATE, G. & ENGBERG, I. (1971). Iontophoretic studies in Deiters' nucleus of the inhibitory actions of GABA and related amino acids and the interactions of strychnine and picrotoxin. *Brain Research* **25**, 431–448.

TOLBERT, D. L., BANTLI, H. & BLOEDEL, J. R. (1976). Anatomical and physiological evidence for a cerebellar nucleo-cortical projection in the cat. *Neuroscience* **1**, 205–217.

TSUKAHARA, N. (1972). The properties of the cerebello-pontine reverberating circuit. *Brain Research* **40**, 67–71.

TSUKAHARA, N., BANDO, T., MURAKAMI, F., & ODA, Y. (1983). Properties of cerebello-precerebellar reverberating circuits. *Brain Research* **274**, 249–259.

WERMAN, R. & GRUNDFEST, H. (1961). Graded and all-or-none electrogenesis in arthropod muscle. II. The effects of alkali-earth and onium ions on lobster muscle fibres. *Journal of General Physiology* **44**, 997–1027.

WILLIAMS, J. T., NORTH, R. A., SHEFNER, S. A., NISHI, S. & EGAN, T. M. (1984). Membrane properties of rat locus coeruleus neurones. *Neuroscience* **13**, 137–156.

YAROM, Y., SUGIMORI, M. & LLINÁS, R. (1980). Inactivating fast potassium conductance in vagal motoneurons in guinea pigs: an in vitro study. *Neuroscience Abstracts* **6**, 198.