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INHIBITORY SYNAPTIC CURRENTS IN RAT CEREBELLAR PURKINJE CELLS: MODULATION BY POSTSYNAPTIC DEPOLARIZATION

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SUMMARY

1. Synaptic currents were recorded in voltage-clamped cerebellar Purkinje cells using the tight-seal whole-cell recording technique. Cells were dialysed with a CsCl solution and were held at −60 or −70 mV. Inhibitory interneurones (basket and stellate cells) were stimulated using an extracellular pipette positioned in the molecular layer. Blockers of excitatory glutamatergic synapses were included in the bath solution.

2. Evoked synaptic currents were observed after a latency of 3–4 ms. The time course of synaptic currents could in most cases be fitted to a biexponential curve, with a rise time constant, τ_{on}, of 1–3 ms and a decay time constant, τ_{off}, of 7–13 ms. These currents were blocked by bicuculline.

3. The mean amplitude of evoked synaptic currents increased in discrete steps when the voltage applied to the stimulating pipette was increased. At each level, very prominent fluctuations of the amplitude were observed among trials.

4. Complex synaptic currents corresponding to repetitive activity of the presynaptic interneurone were occasionally observed, particularly with high intensity presynaptic stimulation. This repetitive activity could lead to bursts of synaptic currents lasting for several seconds.

5. Following a depolarizing voltage train in the postsynaptic Purkinje cell, the amplitude of evoked synaptic currents was first inhibited, and then potentiated. The inhibition was accompanied by a small but consistent increase in τ_{off} and by no alteration in τ_{on}. When using small intensity presynaptic stimuli, it was found that the probability of failures was greatly enhanced. The inhibitory phase lasted for about 1 min before giving way to potentiation. The potentiation returned to the control with a time to half-decay of $12{\cdot}9 \pm 0{\cdot}9$ min.

6. The present results give further evidence to a previously proposed hypothesis that the inhibition produced by Purkinje cell depolarization is mainly presynaptic. The longer lasting potentiation, on the other hand, has most probably a postsynaptic origin.

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INTRODUCTION

Cerebellar Purkinje cells receive inhibitory GABAergic inputs from two classes of interneurones located in the molecular layer (reviewed in Palay & Chan-Palay, 1974; Ito, 1984). Basket cells are closest to the Purkinje cell layer and address their inhibitory signal primarily to the soma and to the main dendrites. Stellate cells are more externally located and have contacts with the more distal part of the dendritic arborization of Purkinje cells. Synaptic currents reflecting the spontaneous activity of these interneurones in acutely prepared slices have recently been recorded using voltage clamp techniques (Konnerth, Llano & Armstrong, 1990; Llano, Leresche & Marty, 1991*a*; Llano, Marty, Armstrong & Konnerth, 1991*b*; Farrant & Cull-Candy, 1991). When using high internal Cl^- solutions, as was usually the case in these studies, the spontaneous synaptic currents have unitary sizes ranging from a few tens of picoamps to several nanoamps at -60 mV. These currents are Cl^--selective and are reversibly blocked by bicuculline. They constitute the most prominent part of the spontaneous synaptic activity in Purkinje cells.

IPSPs have been described *in vivo* in a series of papers on the cat by Eccles and collaborators (Andersen, Eccles & Voorhoeve, 1964; Eccles, Llinás & Sasaki, 1966*a*, *b*; Eccles, Sasaki & Strata, 1967), but a detailed description of IPSPs or IPSCs in slices is still lacking. The first motivation of the present work was to fill this gap. In most experiments, recordings of evoked synaptic currents were obtained in extracellular stimulation of inhibitory interneurones. With this approach, we aimed to establish a more precise characterization of the inhibitory synaptic currents than that achievable by studying the spontaneous synaptic activity. In addition, two specific goals guided our study. Firstly, we were intrigued by the conspicuous variability in size and time course of the synaptic currents, and we have tried to analyse the origin of this diversity. Secondly, a recent study from this laboratory has shown that a direct stimulation of a Purkinje cell leads to complex changes in the response to exogenous GABA applications and in the spontaneous inhibitory synaptic currents (Llano *et al.* 1991*a*). We have now investigated this phenomenon in more detail by using evoked rather than spontaneous synaptic currents.

METHODS

Slice preparation

Sagittal cerebellar slices (140–180 μm thick) were prepared from 9- to 22-day-old rats as described (Llinás & Sugimori, 1980; Edwards, Konnerth, Sakmann & Takahashi, 1989). Briefly, animals were killed by decapitation. A block of cerebellum was quickly removed, was glued on a plastic substrate, and was cut in a chilled chamber with a vibratome. Slices originated from the vermis. After cutting, slices were placed in an oxygenated bath held at 37 °C. They were transferred to the experimental chamber 1–4 h later. In this chamber, the slices were held using nylon threads fixed on a horseshoe-shaped platinum frame.

Recording from Purkinje cells

Slices were observed under Nomarski optics using a Zeiss Axioskop microscope equipped with a 40 × water immersion lens. Purkinje cells were easily visualized. They were 'cleaned' from their glial sheath and from extracellular material using a wide glass pipette (Edwards *et al.* 1989). Then a tight-seal whole-cell recording was obtained with another pipette filled with intracellular solution. For the characterization of evoked synaptic currents, this solution usually contained

150 mM CsCl, 5 mM $MgCl_2$, 0·1 mM $CaCl_2$, 1 mM Cs-EGTA, 10 mM Cs-HEPES, 0·4 mM Na-GTP and 4 mM Na-ATP (pH of 7·3; free Mg^{2+} concentration, 0·6 mM). The high Cl^- concentration of this solution helped to maximize the signal/noise ratio in the study of Cl^--selective synaptic currents. However, in some preparations, spontaneous synaptic currents were so large with this solution that they led to uncontrolled Ca^{2+} spikes and to cell deterioration. To obviate this problem, two different approaches were followed. In some experiments 2 mM QX 314, a blocker of Na^+ channels (Frazier, Narahashi & Yamada, 1970), was included in the recording pipette. Other experiments made use of an alternative solution where half of the Cl^- ions were replaced by 3-(*N*-morpholino)-propanesulphonic acid (MOPS). Such deviations from the standard pipette solution are indicated in figure legends. The pipette access resistance (usually 5–10 MΩ) was compensated to about 60% according to the procedure described in Llano *et al.* 1991*b*. To assess the effectiveness of the voltage clamp, relaxations elicited by 10 mV hyperpolarizing pulses were analysed. After access resistance compensation, these relaxations follow a single exponential with time constant τ'_2. τ'_2 sets the speed of effective voltage control of distal dendrites (Llano *et al.* 1991*b*). τ'_2 values of the cells considered for kinetic analysis ranged between 1 and 4 ms. The experimental chamber was continuously perfused with a saline containing 125 mM NaCl, 2·5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1·25 mM NaH_2PO_4, 26 mM $NaHCO_3$ and 25 mM glucose. This solution was bubbled with a 95% O_2–5% CO_2 mixture which brought the pH to 7·4. Recordings were performed in the presence of 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 100 μM D, L-2-amino-5-phosphonovaleric acid (D, L-APV), both obtained from Tocris Neuramin, except when stated otherwise. These drugs were used to block all excitatory synapses using glutamatergic ionotropic receptors. Tetrodotoxin was purchased from Sigma. All experiments were carried out at room temperature.

Stimulating presynaptic interneurones

Synaptic currents were elicited by extracellular stimulation using glass pipettes filled with saline. In some experiments simple pipettes with tip diameters of the order of 2–8 μm were used. In such cases the reference electrode was a thin platinum wire loosely coiled around the stimulating pipette. In other experiments stimulation pipettes were pulled from double-barrelled 'theta' glass capillaries (Hilgenberg, Malsfeld, FRG). Typical diameter values for each barrel were 10 μm. The stimulus was then applied between the two barrels. Double-barrelled pipettes had a significant interbarrel capacitance, so that it was necessary to prolong the stimulation pulse to 0·3 ms in order to obtain synaptic currents, whereas 50 or 100 μs stimuli were normally used with single-barrelled pipettes. One advantage of double-barrelled pipettes was that the localization of the stimulus was more precise, thus permitting a better mapping of effective stimulation areas. Applied voltages were of the order of 10 to 100 V both with single and with double stimulation pipettes. For presynaptic stimulation, various locations were tried in the molecular layer within the field of view (about 300 μm across). The pipette was positioned so that it nearly touched the surface of the slice. The frequency of stimulation pulses was usually 0·5 Hz. Occasionally frequencies of 0·3 Hz or 1 Hz were used.

Data analysis

Spontaneous currents were recorded on videotape, and were later sampled at 5 kHz after filtering at 1 kHz by an 8-pole Bessel filter. Evoked currents were filtered at 1 kHz, sampled at 12·5 kHz and stored during the experiment. The latency and amplitude of evoked currents were measured using two cursors. The first cursor was placed just before the stimulation artifact and the second at the onset or at the peak of the response. When studying small evoked currents (in TTX, see Fig. 5 below), particular care was taken to reject currents contaminated with spontaneous events to construct amplitude histograms.

Evoked currents were fitted using the program Clampfit (Axon Instruments), and spontaneous synaptic currents were fitted using a program written by Dr S. F. Traynelis.

RESULTS

Location of presynaptic stimulation

Cell bodies 8–10 μm in diameter were observed in the molecular layer and were tentatively identified as inhibitory interneurones. However, we were unable to stimulate unambiguously one of these somata by placing the stimulating pipette in

its immediate vicinity. In a few experiments, the stimulation pipette was moved to map approximately the area within which stimulations gave rise to a given synaptic current. Rather broad areas were found, indicating that successful stimulations are not restricted to cell bodies, and that they involve also (or exclusively) the activation

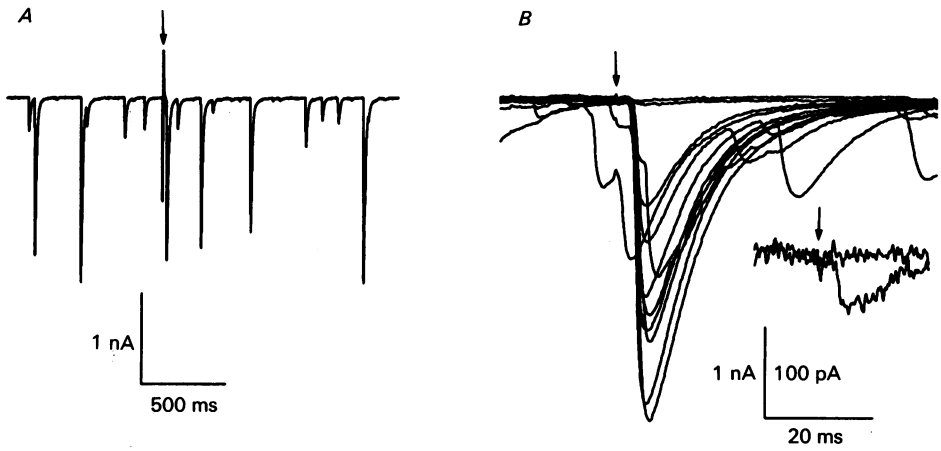

Fig. 1. General features of inhibitory synaptic currents. *A*, slow time scale recording of spontaneous synaptic currents and of an evoked synaptic current (arrow). The deflection coincident with the arrow is the current response to a 10 ms hyperpolarizing pulse of 10 mV amplitude. Such pulses were routinely applied just before stimulating the presynaptic neurone(s) in order to assess the access conductance to the recorded cell and the effective time constant of the voltage clamp, τ_2'. Holding potential, −60 mV. The internal solution contained 75 mM CsCl and 75 mM Cs-MOPS (see Methods). *B*, twelve superimposed traces showing on a faster scale the diversity of responses to identical consecutive presynaptic stimulations (arrow). A trace where no synaptic response was obtained has been used to subtract out the response to the hyperpolarizing voltage pulse and the artifact of the presynaptic stimulation. The inset shows at higher magnification the smallest response out of the twelve, together with a failure. (This failure was taken outside of the twelve consecutive traces. There was also one failure in the sequence, but the trace was contaminated by a preceding spontaneous event and is therefore not shown in the high-magnification inset.)

of neurites. Two kinds of results were obtained. In some cases successful stimulation occurred only in a narrow band (approximately 20 × 200 μm) flanking the Purkinje cell layer. Alternatively, a wider area straddling the entire molecular layer was found. The first type of presynaptic stimulation area corresponds to the location of the axons of basket cells, which run in this narrow area before making contact with Purkinje cells. Thus such stimulation fields probably reflect the activation of one or several basket cell axons. The second type of stimulation area (a rectangle across the molecular layer, with a radial long axis) presumably corresponds to the stimulation of the dendritic tree of either a basket cell or a stellate cell.

The most reliable method of obtaining robust evoked GABAergic synaptic currents was to place the stimulating pipette close to the Purkinje cell layer, at a distance of 30–60 μm from the recorded Purkinje cell, in order to stimulate basket cell axons. The majority of the experiments presented below were obtained under these conditions. However, very similar results were obtained with more distant stimulation (as far as 250 μm from the recorded cell) or with stimulating spots located more externally in the molecular layer. Still more distant stimulations were

not attempted due to practical limitations imposed by the size of the field of view. For comparison, lateral spread of interneurone-mediated inhibitory IPSPs has been found to extend as far as 1 mm in the cat (Eccles *et al.* 1967). Finally, stimulation close to the recording site (< 30 μm) led to the direct stimulation of the recorded cell and were therefore impractical.

General features of evoked synaptic currents

The main features of evoked synaptic currents are illustrated in Fig. 1. Between stimulations, many spontaneous synaptic currents having peak amplitudes ranging from 10 pA or less to several nanoamps were observed (Fig. 1*A*). Superimposed on this activity, evoked synaptic currents with similar amplitudes and time course were found. Evoked currents following a series of twelve consecutive stimulations are displayed in Fig. 1*B*. In one out of the twelve traces, there was no signal to be seen. In the other traces, the amplitude of the evoked currents varied on a very wide range, from 50 pA to 3·5 nA. On inspection of the entire recording, it was clear that this extremely wide variation could not be explained by an interference between evoked and spontaneous activity. Thus the 50 pA events such as that shown in the inset of Fig. 1*B*, were much too frequent to be due to the random occurrence of spontaneous synaptic currents. It was not possible to correlate the observation of particularly small (or large) evoked currents with the previous occurrence of spontaneous currents.

CNQX (10 μM) blocks entirely the excitatory currents elicited in Purkinje cells by parallel fibre or by climbing fibre activation (Llano *et al.* 1991*b*). Thus the presence of CNQX in the bath ensured that all excitatory inputs to the Purkinje cell were blocked. Because there is evidence indicating that the excitatory input of parallel fibres onto interneurones involves both NMDA and non-NMDA glutamatergic receptors (Llano *et al.* 1991*b*; I. Llano & H. M. Gerschenfeld, manuscript in preparation), 100 μM APV was also included in the external solution. In the presence of both CNQX and APV, the other pathway likely to remain functional in the molecular layer is the monosynaptic connection between GABAergic inhibitory interneurones and Purkinje cells. Indeed bath application of 15 μM bicuculline led to a total abolition of both evoked and spontaneous synaptic currents. The effects of bicuculline (which were reversible upon washing) indicate that evoked currents are due to activation of $GABA_A$ receptors. Evoked and spontaneous synaptic currents inverted near 0 mV when using an isotonic Cl^- internal solution.

Kinetics of evoked currents

Kinetics of evoked currents were studied in cells having a fast capacitive transient, with a time constant $\tau'_2 < 4$ ms, thus ensuring optimal voltage clamp conditions (see Methods). The onset of synaptic currents is illustrated in Fig. 2 on a fast time base for the same experiment as that of Fig. 1. Many events (53% in this particular experiment) display an inflexion in their rising phase (Fig. 2*A*), indicating that they contain two or more components with different latencies. A statistical analysis showed that these multiple events were too frequent by a factor of about 10 to be accounted for by the superimposition of spontaneous activity and simple evoked currents. The latency histogram from the same experiment (Fig. 2*B*) displayed a rather large scatter. The variability of the latencies illustrated in Fig. 2 is unlikely

to be due to a polysynaptic pathway since all experiments were performed in the presence of CNQX and APV, under conditions where all excitatory synapses of the cerebellar cortex are presumably blocked. We will see below evidence suggesting that some of the longer latencies are due to repetitive firing of presynaptic neurones.

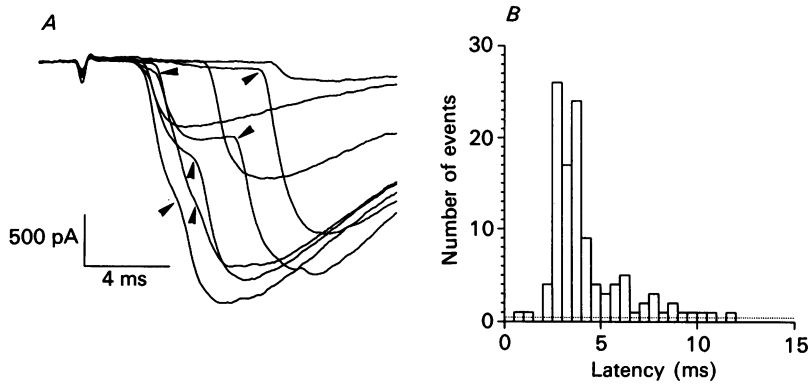

Fig. 2. Variability in onset kinetics. *A*, selected examples of evoked currents, from the same experiment as in Fig. 1. Many currents have a clear inflexion (arrow-heads) in their rising phase. *B*, latency histogram from the same experiment. Latencies (total number of events, 112) were measured to the first clear deflection from the baseline, with a threshold of 20 pA. When multiple events were encountered, additional latencies were entered at the inflexion points of the current. Time 0 is the onset of the stimulation artifact. The dotted line represents the number of events per bin expected on the basis of spontaneous activity.

Finally, it should be noted that the spontaneous activity, which was rather high in this experiment, must have contributed some events in the latency histogram. Given the frequency of the spontaneous activity between simulations, a contribution of 0·47 events per bin was predicted. This is drawn as the dotted line in Fig. 2*B*. It can be seen that the data points exceed this level between 2 and 10 ms. Within this time window, the average latency was $4{\cdot}11 \pm 1{\cdot}59$ ms (mean $\pm$ S.D., $n = 106$). When restricting the analysis to first latencies, the average dropped somewhat to $3{\cdot}64 \pm 1{\cdot}46$ ms (mean $\pm$ S.D., $n = 67$). Mean first latency values from three other experiments ranged from 2·8 to 3·5 ms. In all cases scattered latencies and examples of multiphasic rises were observed. However, the proportion of complex rising phases was somewhat lower in other cells (ranging between 16 and 22%) than in that of Figs 1 and 2 (with 53% of complex events).

If events with complex rising phases were rejected from the analysis, most of the remaining evoked synaptic currents could be fitted to the sum of two exponentials with opposed signs (Fig. 3, left panel). In the cell illustrated in Figs 1–3, the time constant of the rising phase, τ_{on}, had an average value of $1{\cdot}4 \pm 0{\cdot}25$ ms (mean $\pm$ S.D., $n = 15$). The time constant of decay, τ_{off}, averaged $8{\cdot}3 \pm 4{\cdot}3$ ms. In other cells, τ_{on} and τ_{off} had similar values to those found here. When gathering results from four cells, τ_{on} averaged $1{\cdot}65 \pm 0{\cdot}78$ ms (mean $\pm$ S.D., $n = 4$), and τ_{off} averaged $9{\cdot}3 \pm 2{\cdot}7$ ms. In one of these cells, about one-fourth of the evoked currents had a more complicated decay than that illustrated in Fig. 3. In these records, a total of three exponential

components (one for the rising phase and two for the decay) were required to fit the time course of the synaptic currents. They were excluded from the estimate of τ_{off} for that cell.

Evoked current as a function of intensity of presynaptic stimulation

As the intensity of the presynaptic stimulation was increased, several levels of activity could be distinguished in the synaptic currents (Fig. 4). Below a certain

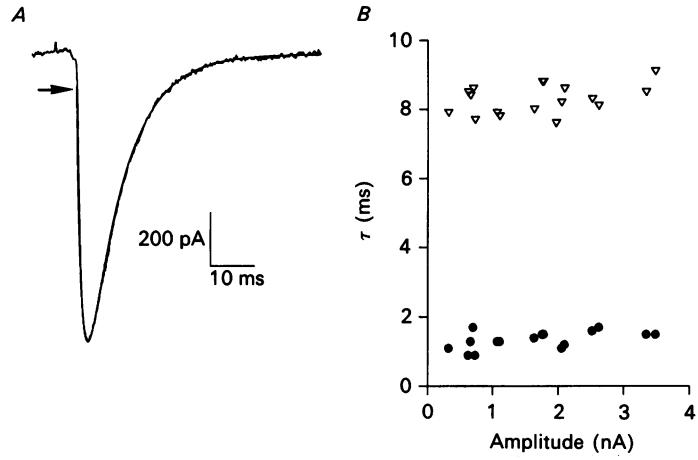

Fig. 3. Kinetics of evoked synaptic currents. *A*, evoked synaptic current with superimposed double exponential fit. The fitted curve had time constants $\tau_{on} = 1{\cdot}3$ ms, $\tau_{off} = 7{\cdot}9$ ms. The fitted portion of the data points starts at the arrow. *B*, τ_{on} (●) and τ_{off} (▽) values for a number of fits such as shown on the left, as a function of synaptic current amplitude. Same cell as in Fig. 1. Time constant of capacitive current during recording, $\tau'_2 = 2{\cdot}1$ ms.

threshold no response at all was obtained. Then a range was encountered where failures alternated with evoked currents of about 50 pA. When increasing the presynaptic stimulus further, successive levels with rather stable mean current amplitudes were uncovered. In the example shown, successive plateau levels with mean amplitudes of approximately 50, 800 and 1200 pA can be distinguished. In each plateau region, large fluctuations of individual synaptic current were observed around the mean.

The variability of the evoked current amplitudes which is observed in such experiments is quite remarkable. Neither climbing fibre nor parallel fibre stimulation produces a comparable variation of the evoked excitatory synaptic currents recorded previously in the same preparation (Konnerth *et al.* 1990; Llano *et al.* 1991*b*). On the other hand, amplitude variations up to 200 pA have been reported in GABAergic inhibitory synaptic currents recorded in granule cells of rat hippocampal slices (Edwards, Konnerth & Sakmann, 1990). In the latter study equally spaced peaks could be distinguished in the amplitude histogram of evoked synaptic currents, and it was suggested that the interpeak interval (7–18 pA at −50 mV, using a high internal Cl^- solution) represented the quantal size. Regularly spaced peaks were not

apparent in our Purkinje cell recordings. This is possibly due to technical limitations linked with the large size of these cells, as further discussed below, or alternatively to the relatively small number of events (155) used for the histogram. Assuming a quantal size of the order of 10–20 pA in the present experiments, could the variation

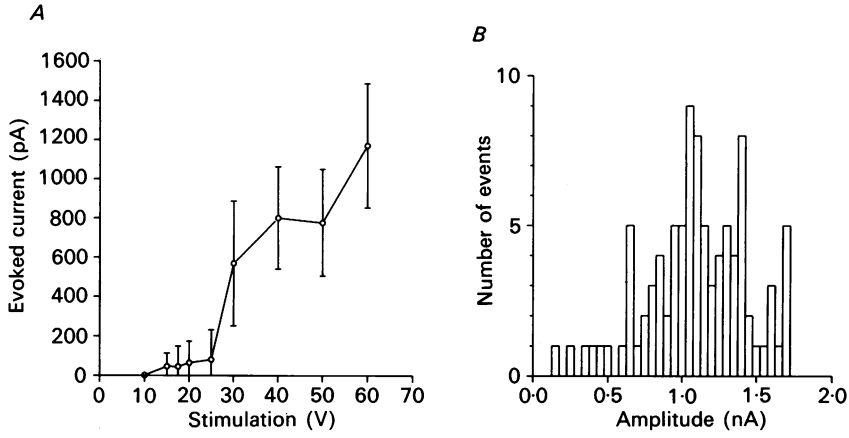

Fig. 4. Amplitude of synaptic current as a function of stimulation voltage. *A*, mean values ± S.D. ($n = 10$–27) of synaptic currents recorded at −70 mV are plotted as a function of voltage applied in the stimulation pipette. Below 10 V, no synaptic current response was detected. *B*, amplitude histogram from the same experiment obtained with 60 V stimuli. 93 events were used for this histogram.

of the synaptic current amplitudes simply reflect the random fluctuations of the quantum content expected from independent release sites? To approach this question, a variance analysis was performed on data such as that illustrated in Fig. 4. Let us assume for simplicity that all release sites have the same probability, p, to release a quantum, that release sites are independent, and that the release of a quantum results in a fixed current, q. Under these hypotheses, the ratio of the variance over the mean of the currents will equal q, the quantal size. The values found for this ratio were very large. Thus the records obtained with a stimulation intensity of 20 V in the experiment shown in Fig. 4 yielded a variance to mean current ratio of 190 pA. This is much larger than the quantal size (10–25 pA) expected from the hippocampal slice results when taking into account the difference in driving force between the two experimental conditions (−70 *versus* −50 mV). The discrepancy indicates either that the quantal size is larger in Purkinje cells, or that the release sites were not independent in the present experiment. (It can be shown that any heterogeneity of p among release sites would only worsen the discrepancy.) In order to decide between these possibilities, additional experiments were performed in the presence of low concentrations of TTX (10–30 nM). Under these conditions, the spontaneous inhibitory currents were reduced considerably both in amplitude and in frequency. Evoked synaptic currents were also reduced. Variance analysis of these currents revealed variance/mean current ratios much lower than in the control (19 pA in the experiment of Fig. 5). The latter results do not support the view that

the quantal size of inhibitory currents in Purkinje cells would be exceptionally large. Figure 5 illustrates an amplitude histogram obtained in the presence of a low dose of TTX. There is a peak about 10 pA wide around 0 pA amplitude which presumably reflects failures. This is followed by a broad component ranging from 10 to 100 pA,

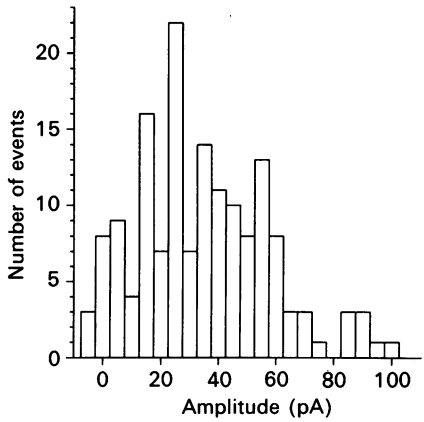

Fig. 5. Amplitude histogram of partially blocked synaptic currents. TTX (20 nM) was used to reduce the size of synaptic currents. Holding potential, −70 mV. 155 events were used to build this histogram. The stimulation intensity and frequency were respectively 8 V and 1 Hz.

where it is impossible to distinguish regularly spaced peaks. Such results are clearly incompatible with a large quantal event of several tens of picoamps. They leave, however, entirely open the possibility of a smaller quantal size (15 pA or lower in the present case).

Spontaneous synaptic currents

Spontaneous synaptic currents were analysed in some of the recordings. Amplitude histograms showed a wide scatter and covered the same range as that found for evoked currents (see Llano *et al.* 1991*a* for examples of histograms of spontaneous synaptic currents). Kinetic properties were similar to those of evoked currents, with one difference (Fig. 6). For a given cell, the scatter of both τ_{on} and τ_{off} values was much more pronounced for spontaneous events than for evoked currents, as may be seen by comparing the results in Fig. 3 and in Fig. 6. As a rule, τ_{on} values covered the entire range between 0·5 ms and τ'_2. Thus the spread of τ_{on} was much larger in cells which had a fast compensated capacitive current (low τ'_2) than in cells with a slower capacitive current. τ_{off} varied significantly from cell to cell, with mean values ranging between 6·2 and 13·6 ms. The average of mean τ_{off} values from five cells with fast capacitive transients ($\tau'_2 < 2$ ms) was $8{\cdot}8 \pm 3{\cdot}0$ ms. This is close to the corresponding value for evoked currents ($9{\cdot}3 \pm 2{\cdot}7$ ms, see above). There was no correlation between τ_{on} and τ_{off}, or between τ_{on} and the peak amplitude of spontaneous synaptic currents. These results indicate that there is no difference in size or time course of decay between somatic (as identified by a fast τ_{on} value) and dendritic (with slower τ_{on}

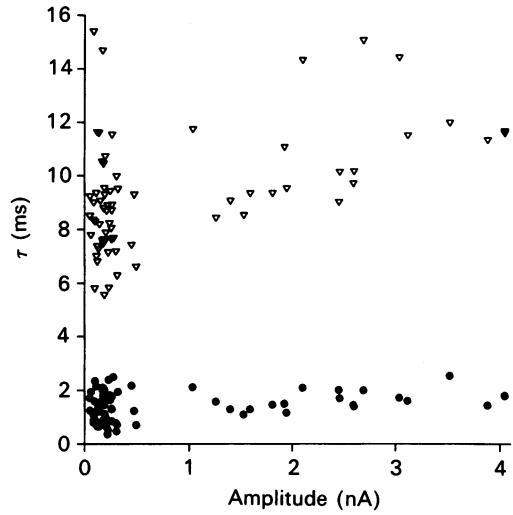

Fig. 6. Time course analysis of spontaneous synaptic currents. Biexponential fits were performed on sixty-nine spontaneous events recorded in a single cell over a 54 s period. τ_{on} (●) and τ_{off} (▽) are plotted as a function of peak current amplitude. Holding potential −70 mV. $\tau_2' = 1{\cdot}9$ ms.

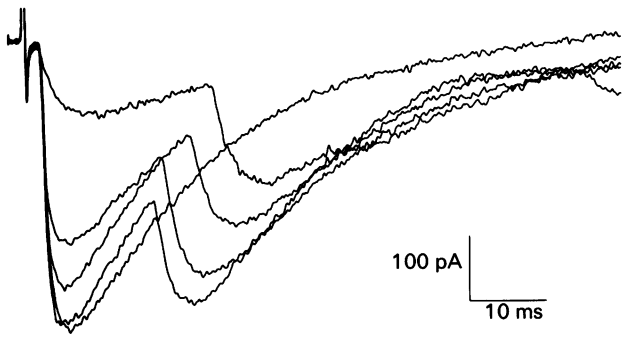

Fig. 7. Double synaptic events. Superimposed records of synaptic currents with a simple decay or with a rebound synaptic event. Holding potential, −80 mV; 10 nM TTX in bath. Stimulation intensity, 25 V.

values, close to τ_2') synaptic currents. Because basket and stellate cells mainly contact somatic and dendritic areas respectively, the results further indicate that the two cell types make similar functional connections onto Purkinje cells.

Bursts of synaptic currents

In certain cells it was found that a few successive synaptic currents were elicited by a single presynaptic stimulus, with a large variability between trials. An example where either single or double synaptic currents were obtained with approximately the same likelihood is shown in Fig. 7. In this experiment, where 10 nM TTX was employed, the spontaneous synaptic activity was rather low (about one event of

> 100 pA in 300 ms) such that the appearance of the secondary synaptic current cannot be due to the chance events linked to spontaneous synaptic currents. In other cells, the multiple character of the evoked response was much more prominent. In one such recording, illustrated in Fig. 8*A*, single presynaptic stimuli elicited bursts

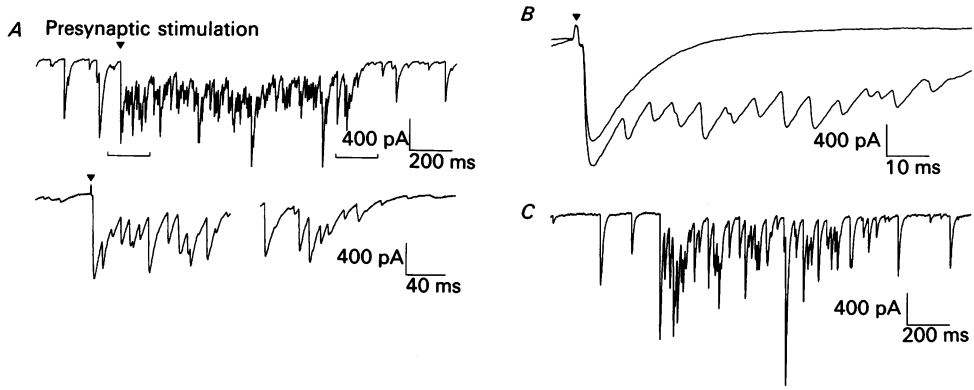

Fig. 8. Bursts of synaptic activity. *A*, the upper trace shows a burst (duration 1·2 s) of synaptic activity triggered by a single presynaptic stimulation (▼). Lower traces, onset and end of burst displayed on a faster time scale. The traces correspond to the two periods indicated below the upper trace. Holding potential, −60 mV; 2 mM QX 314 in internal solution (see Methods); no TTX in external solution. *B*, superimposed responses showing a single synaptic event and the beginning of a burst. Same experiment as in *A*. The stimulus intensity was set at the threshold for eliciting a burst. *C*, burst of spontaneous synaptic currents, from another cell. Standard intracellular solution. The bath solution was normal saline without CNQX or APV. Holding potential, −70 mV.

lasting from 0·33 to 1·2 s. Within a burst, individual synaptic currents appeared to have rather homogeneous rise time and decay time constants (Fig. 8*A*). An autocorrelation analysis of the burst shown in Fig. 8 gave a periodic signal with five equidistant peaks indicating a frequency of 105 Hz. These observations are consistent with the notion that the burst is due to repetitive firing of a single neurone. The onset and end of the bursts were equally abrupt (Fig. 8). What exactly determines the appearance of multiple or bursting synaptic currents could not be decided in the present experiments. When present, bursts had a specific threshold which was higher than that required to obtain single synaptic currents. Thus in experiments where bursts were observed, it was possible to revert to simple synaptic events simply by decreasing the strength of the stimulus. Figure 8*B* illustrates that simple events or bursts could be randomly elicited near the threshold for burst activation. Bursts of spontaneous synaptic currents were also observed occasionally, particularly on slices of young animals (less than 14 days). A particularly striking example is illustrated in Fig. 8*C*. As in the case of the burst of Fig. 8*B*, that of Fig. 8*C* gave a periodic autocorrelation function with an interpeak period corresponding to a frequency of 50 Hz. This particular recording was obtained without CNQX and APV. In addition to the dramatic, but rather rare, cases of long bursts exemplified in Fig. 8*C*, spontaneous synaptic currents appeared to display frequently shorter bursts of a few events.

Effects of postsynaptic depolarizing voltage pulses

Depolarizing voltage pulses applied to Purkinje cells elicit a dual inhibitory–excitatory effect on GABAergic currents (Llano *et al.* 1991*a*). Previous evidence indicates that both effects depend on Ca^{2+} entry into Purkinje cells, that the

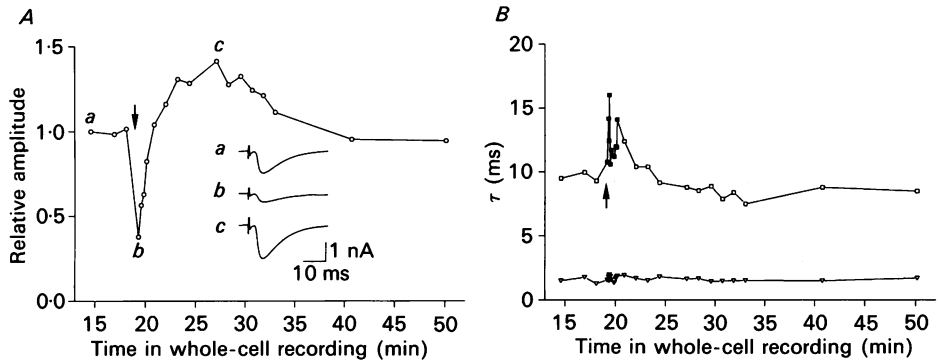

Fig. 9. Inhibition–potentiation sequence of synaptic currents following a postsynaptic depolarizing train. Synaptic currents were measured before and after applying a train of eight 100 ms voltage pulses to 0 mV (arrow). The pipette solution contained 75 mM CsCl and 75 mM Cs-MOPS. *A*, average amplitudes (from eight to thirty-two trials) as a function of time. The mean amplitude is first depressed to 38% of the control, but this inhibition is soon followed by a potentiation to 142% of the control. Average traces obtained in the control (*a*), at the peak of the inhibition (*b*) and at the peak of the potentiation (*c*) are displayed below the plot. *B*, time constants of onset (τ_{on}, triangles) and decay (τ_{off}, squares) of the synaptic currents as a function time. For kinetic analysis, only evoked currents free from contaminating spontaneous activity were considered. Before 18 and after 21 min of whole-cell recordings, up to twenty such currents were averaged for kinetic analysis (open symbols). Just after the train (at 19 min, indicated by arrow), however, fits to individual currents were performed (filled symbols).

inhibition is short lasting and presynaptic, and that the potentiation is more sustained and postsynaptic (Llano *et al.* 1991*a*). In the present work the same voltage stimulation protocol (eight pulses of 100 ms duration to 0 mV at 0·5 Hz) which previously proved to transiently inhibit the spontaneous synaptic currents and to potentiate GABA-evoked currents was applied to Purkinje cells. This resulted in a sequence of inhibition and of potentiation of evoked synaptic currents (Fig. 9). Out of eleven cells tested, eight showed a clear cut potentiation (whereas all cells displayed an initial inhibition). Four of these cells, including that of Fig. 9, had a sufficiently stable control and a sufficiently long after-pulse period to allow a quantitative analysis of the results. On average, the peak amplitude of the potentiated currents was $146{\cdot}7 \pm 8{\cdot}2\%$ (mean $\pm$ S.D., $n = 4$) of the control. This is in excellent agreement with the figure of $144 \pm 24\%$ found earlier using exogenous GABA applications (Llano *et al.* 1991*a*). The time to half-recovery (measured from the time of the train) was $12{\cdot}9 \pm 0{\cdot}9$ min (mean $\pm$ S.D., $n = 3$), compared to a value of $3{\cdot}7 \pm 1{\cdot}5$ min found earlier using GABA applications. Thus the half-recovery time is longer in the present series of experiments. On the whole, however, the results are consistent with the hypothesis that the potentiation of evoked synaptic currents and

of GABA-induced exogenous currents reflect the same underlying mechanisms. Additional analyses, not detailed here, showed that the spontaneous synaptic currents were potentiated in parallel with the evoked currents.

The inhibitory effect lasted for a total period of about 1 min after the end of the train. Given the uncertainty concerning the mechanism of this modulation, a special

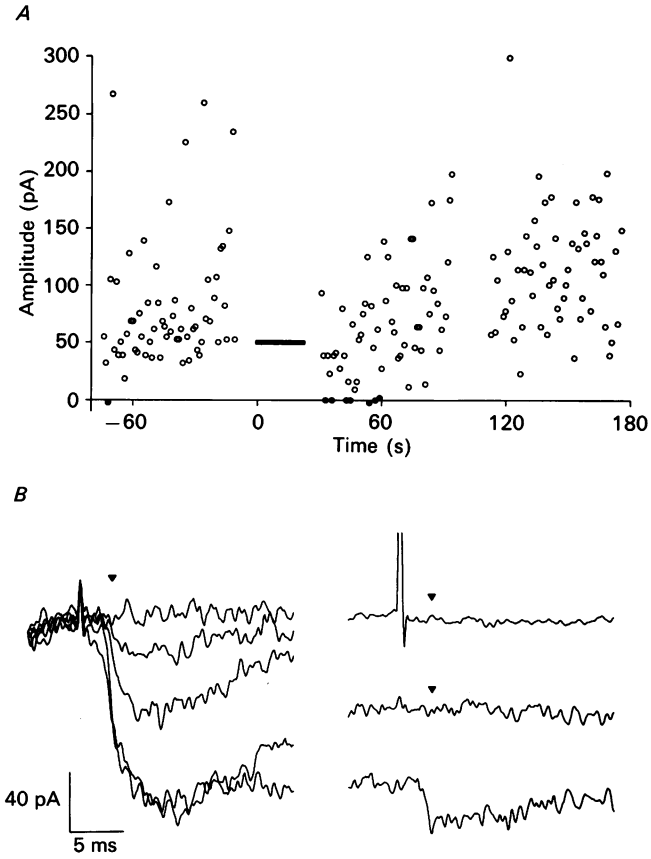

Fig. 10. Reduction of synaptic efficacy by postsynaptic stimulation. *A*, the amplitude of synaptic currents is plotted as a function of time before and following a train of eight depolarizations to 0 mV lasting 100 ms each, applied to the Purkinje cell. Events considered as failures (< 10 pA) are plotted as filled symbols. Holding potential, −60 mV. Frequency of presynaptic stimulation, 1 Hz. Intensity of presynaptic stimulation, 11 V. Presynaptic stimulation was interrupted for about 30 s around the time of postsynaptic stimulation (bar). Note the large increase of failure frequency after the train. Stimuli were interrupted near 100 s to examine the passive electrical properties of the cell. *B*, sample current responses before (left) and after (right) postsynaptic train. Data were taken from the same cell as in *A*, but from another trial. Failures are indicated by arrow-heads near the time when synaptic currents, if present, have their rising phase. In the control (left) a single failure is obtained in the five trials displayed. After the train (right) the frequency of failures increased dramatically. An example of failure is shown in the middle trace, right. The uppermost trace on the right shows the average of ten traces classified as failures. This average was used to subtract the stimulus artifact from all other traces shown. The lowest trace on the right shows a typical IPSC after the train.

effort was made to study this inhibition in a more detailed manner than was previously achievable using spontaneous synaptic currents. The first question which was examined was whether the inhibition was restricted to a particular subset of inhibitory inputs. This was clearly not the case, since synaptic currents were reliably

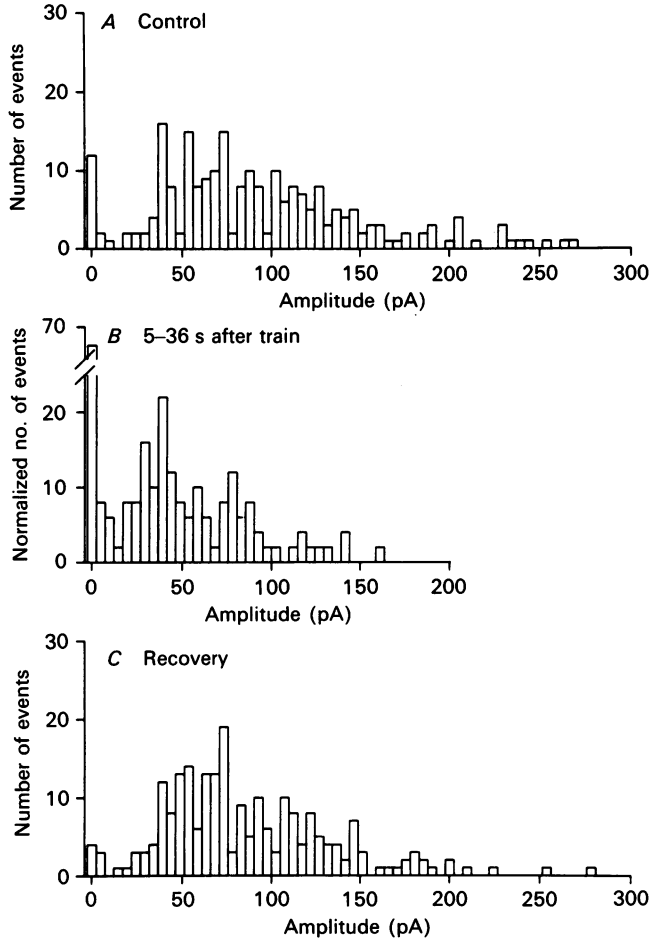

Fig. 11. Effect of postsynaptic stimulation on amplitude histogram of synaptic currents. Amplitude histograms were collected from four successive runs such as illustrated in Fig. 10 (same experiment). Control and recovery periods contain sixty-four amplitude values per run, while the after-train periods contain thirty-two values. The amplitudes of the after-train histogram have been normalized such that the total number of entries are the same in all histograms. Recovery periods start approximately 75 s after the end of the trains.

reduced in all recordings regardless of the location of the stimulation pipette. (However, cells which had a leakage current in excess of 500 pA at -70 mV often failed to display the inhibitory effect. This is presumably because such cells have abnormal internal Ca^{2+} levels.) Similar reductions could be observed when using small or large intensity presynaptic stimulations.

Figure 9 illustrates an example from a cell with a large amplitude control response.

Following the depolarizing voltage train, the response was reduced to 38% of the control. During the inhibition, τ_{off} increased somewhat compared to the control, whereas τ_{on} was unchanged. On average, the peak amplitude decreased to 36 ± 16% of the control (mean $\pm$ S.D., $n = 5$), and τ_{off} increased to 132 ± 11% of the control. The potentiation phase, on the other hand, was not accompanied by any significant kinetic change in the evoked synaptic current. The plot of Fig. 9*B* shows that there was no alteration of τ_{on} or τ_{off} during this phase in the experiment shown – a result consistent with those obtained in other cells.

Figure 10 illustrates short-term inhibition as observed following low intensity stimulations. The main effect here was that the number of failures increased markedly following the pulses. Examples of failures (indicated by arrow-heads) before and after a train are shown in Fig. 10*B*. In the uppermost trace of Fig. 10*B*, right, ten traces classified as failures have been averaged together. There is no IPSC-like signal to be recognized in the average. It can be estimated that any residual IPSC in the average must be smaller than 2 pA. As 2 pA is roughly the size of one single GABA-activated channel under the present experimental conditions (Bormann, Hamill & Sakmann, 1987; Llano, Gähwiler & Marty, 1992), it appears that a majority of the traces classified as failures do not involve a single GABA-sensitive channel activation, and are therefore genuine failures. Amplitude histograms obtained from the same experiment are shown in Fig. 11. It may be seen that there was a 5·6-fold increase in the number of failures following voltage pulses in this cell. This increase was reversible and reproducible. (Results from four successive runs have been summed to construct the amplitude histograms of Fig. 11.) The strong enhancement in failure frequency apparent in this experiment (representative of three) strongly reinforces the view that the Ca^{2+}-recruiting voltage pulses exert their effects presynaptically. Note that no potentiation is apparent at late times in the plot of Fig. 10. This is because several trains were given at short intervals in order to sum amplitude histograms. These stimulations were too frequent to allow the potentiation mechanism to return to its resting state. Following the first train in the series, however, a clear late potentiation was observed.

DISCUSSION

The general features of the GABAergic synaptic currents in Purkinje cells as described in the present work are similar to those of hippocampal granule cells as reported by Edwards *et al.* (1990). In both cases, the synaptic currents have a rather slow decay, with a half-decay time on the order of 10 ms. Nevertheless several differences may be noted between the two preparations. First, the rise time of the currents in Purkinje cells appears to be slower (by a factor of about 2–4) than in the hippocampus. Second, the decay phase can be fitted reasonably well by a single exponential in the Purkinje cells, whereas two distinct components could be distinguished in the hippocampal granule cells (Edwards *et al.* 1990). Third, regularly spaced peaks were distinguished in the hippocampal recordings, whereas no such peaks could be consistently obtained in the present study. These three sets of differences may be explained to some extent by the large size of the Purkinje dendritic arborization and to the unavoidable distortion which is introduced by

passive electrical properties for distal synaptic inputs. Such effects will slow down the synaptic current rise, will erode any fast component of the current decay, and might broaden the width of quantal peaks such that adjacent peaks become more difficult to separate.

The quality of voltage control has been analysed in this preparation by Llano *et al.* 1991*b*. Briefly, it was found that, for distal synapses, the speed of the voltage clamp is determined by the time constant of decay of the capacitive current, τ_2', as obtained after series resistance compensation. In the best cases, as exemplified in Fig. 1, τ_2' was between 1 and 2 ms. In the experiment of Fig. 1*A*, for example, τ_2' was 2·1 ms. In the same experiment, the rise time of synaptic currents, τ_{on}, ranged from 0·9 to 1·7 ms. These results are consistent with the proposal that τ_{on} is largely determined by passive cell properties. On the other hand, the observation that many synaptic currents displayed detectable inflexions in their rising phase (Fig. 1*B*), and that the delays were variable between trials, suggest that part of the rise time of synaptic currents may alternatively be ascribed to asynchrony among unresolved components of the synaptic current.

Because the time course of decay, τ_{off}, was much larger than τ_2' in the best recordings, the decay kinetics of synaptic currents must have been faithfully recorded (Llano *et al.* 1991*b*). Thus the discrepancy between the values of τ_{off} found here (9·3 ± 2·7 ms) and the two time constants found in hippocampal granule cells (2·2 and 66 ms: Edwards *et al.* 1990) must reflect genuine differences between the two preparations. In hippocampal pyramidal cells, Ropert, Miles & Korn (1990) found still a different situation, with a single component decay having a time constant of 25 ms. One possibility is obviously that the cell types involved in these various studies express different subsets of subunits composing the GABA-sensitive channels.

The variability among successive evoked synaptic currents was a striking feature of GABAergic currents in Purkinje cells. For reasons which have been discussed above, the variations cannot be attributed to random variations among independent release sites. Because several inhibitory interneurones contact a single Purkinje cell, it seemed possible that the variation reflected changes in the number of recruited presynaptic interneurones. In such a case, one would expect to obtain more homogeneous synaptic currents with minimal stimulation intensities, for which only the cell with lowest activation threshold would occasionally be stimulated. Contrary to this expectation, however, very diverse amplitudes were obtained near the threshold of the response (see Fig. 10*A* for an example). A second argument against the multi-cell hypothesis stems from the very large range of the response amplitudes. For the sake of the argument, let us consider an experiment (such as that illustrated in Fig. 1) where basket cell axons were stimulated. In the rat, there are six to ten basket cells contacting one Purkinje cell (Palay & Chan-Palay, 1974). Probably nearly half of these cells are destroyed when cutting the slice. Of the remaining neurones, one half (up to three only) fall on the same side of the Purkinje neurone as the stimulating pipette and are therefore possible presynaptic neurones. This low number has now to be related to the many levels of synaptic amplitudes elicited in recordings such as those of Fig. 1, where values ranging continuously from 50 to 3500 pA were found. Assuming for the sake of simplicity that all cells give the same individual synaptic current, the ratio (3500/50 = 70) should be equal to the number

of presynaptic neurones. The discrepancy between this figure and the upper estimate of three obtained from anatomical results makes the multi-cell hypothesis very unlikely.

As an alternative to the multi-cell hypothesis, it may be proposed that the presynaptic stimulation could invade varying parts of the axonal arborization of inhibitory interneurones as a result of random propagation failures at branching points. This hypothesis would be compatible with the large variance of the low amplitude synaptic currents and with the large range of response amplitudes. While the number of active presynaptic cells would be fixed for a given stimulation intensity, this number may increase stepwise with this intensity, generating multi level stimulation/amplitude curves as illustrated in Fig. 3. In summary, the variability of synaptic currents may reflect the rather peculiar conditions used in the present work, where presynaptic neurites were directly stimulated. It is interesting that intracellularly recorded IPSPs elicited polysynaptically by stimulation of parallel fibre afferents do not show large variations from one trial to the next (Eccles *et al.* 1966*b*). This may be because all presynaptic terminals are equally activated following the firing of the soma of an interneurone. One consideration which further complicates the interpretation of the results is the fact that axons of presynaptic basket cells form a complex structure (the pinceau) around Purkinje cell axons. Basket cell axons are linked by inhibitory axo-axonal synapses and by gap junctions in these structures (Palay & Chan-Palay, 1974). It is unclear at present whether and how these junctions might contribute to the synaptic current variability.

The repetitive mode of synaptic discharge seen in Figs 7 and 8 is analogous to that reported for GABAergic synaptic currents in thalamic neurones (Leresche, 1992). The mechanism underlying such effects is unknown. If one accepts that GABAergic synapses are inhibitory (see, however, the review by Cherubini, Gaiarsa & Ben-Ari, 1991, for exceptions to this rule in the hippocampus) it appears unlikely that the multiple synaptic currents would be generated by a polysynaptic mechanism, because the bursts could be obtained in the presence of CNQX and APV (Figs 7 and 8*A* and *B*). The alternative, more likely explanation is that the bursts are due to repetitive firing of a presynaptic neurone. In such a view the bursts would simply reflect an endogenous tendency of presynaptic neurones to fire repetitively. Such a suggestion is in line with previous evidence using extracellular recordings *in vivo* that showed repetitive discharges of up to six action potentials of interneurones following a short stimulation of parallel fibre inputs (Andersen *et al.* 1964; Eccles *et al.* 1966*a*).

The present results confirm and extend a previous report that depolarizing pulses in Purkinje cells lead to complex changes in GABAergic currents (Llano *et al.* 1991*a*). Whereas this earlier study was devoted to spontaneous synaptic currents and to GABA-evoked currents, the present work shows that evoked synaptic currents are also modulated. No physiological role for changes of the synaptic efficacy of inhibitory interneurones in the cerebellum is known. It can be foreseen, however, that the inhibition–potentiation sequence uncovered here should strongly alter the efficacy of the endogenous inhibitory barrage of GABAergic interneurones and hence the probability of firing of a Purkinje cell. Assuming a constant and moderate frequency of excitatory inputs (e.g. from parallel fibres), one would expect an enhancement of firing during 1 min, followed by a decreased activity over a period

of time of about 20 min. This should result in periodic bouts of activity of the Purkinje neurone. However, such periodic changes may be impossible to observe in slices due to the abnormal condition of afferent inputs. One interesting finding of the present study is that the duration of the potentiation found here is about 3 times longer than in Llano *et al.* 1991*a*. This suggests that rather minor changes in experimental conditions may alter significantly the duration of potentiation. One difference with earlier experiments concerns the Mg^{2+} concentration of the pipette solution. In the earlier work by Llano *et al.* there was a very low concentration of free Mg^{2+} ions in this solution as the concentration of ATP exceeded the total Mg concentration. In the present work, however, the internal solution contained 0·6 mM free Mg^{2+}.

The present work adds little to the elucidation of the actual mechanisms of the inhibitory–potentiating effects. Nevertheless, the finding that the frequency of failures is strongly increased during the inhibitory phase reinforces previous evidence indicating that the underlying mechanism is mainly presynaptic (Llano *et al.* 1991*a*). The fact that the time constant of decay of synaptic currents was somewhat increased during the inhibitory phase (Fig. 9) does not contradict this conclusion. If the inhibition takes place in the presynaptic terminals, it may be that the presynaptic depolarization is slower and less synchronized following the train than in the control. This could lead to a prolonged synaptic current without implying any postsynaptic change. The late potentiation, on the other hand, is most probably postsynaptic, as indicated by the earlier finding of a corresponding potentiation in responses to exogenous GABA applications (Llano *et al.* 1991*a*).

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