

Synaptic currents in cerebellar Purkinje cells

(climbing fiber/parallel fiber/glutamate receptors/brain slices/patch clamp)

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ABSTRACT Cerebellar Purkinje cells are known to receive strong excitatory input from two major pathways originating outside the cerebellum and inhibitory input from two types of neurons in the cerebellar cortex. The functions and synaptic strengths of these pathways are only partially known. We have used the patch-clamp technique applied to Purkinje cells in thin slices of rat cerebellum to measure directly the postsynaptic currents arising from the two major excitatory pathways and one of the inhibitory inputs. Inhibitory synaptic currents occur spontaneously with high frequency and are variable in amplitude, ranging, in our recording conditions with high internal Cl^- , from less than 100 pA to more than 1 nA. These currents are blocked by the γ -aminobutyrate type A antagonist bicuculline. One of the excitatory inputs is all or none. For threshold stimulation, the synaptic current is either full amplitude, when the presynaptic fiber is successfully stimulated, or completely absent. This synaptic current is often larger than 1 nA and is virtually eliminated by 2 μM 6-cyano-7-nitroquinoxaline-2,3-dione, a blocking agent thought to be specific for glutamate receptors that are not of the *N*-methyl-D-aspartate type. Its all-or-none character identifies it as arising from a climbing-fiber synapse. The other excitatory input produces a synaptic current that is smoothly graded as a function of stimulus intensity. This response we believe arises from the stimulation of mossy fibers or granule cells. The synaptic current associated with this input is also largely eliminated by 2 μM 6-cyano-7-nitroquinoxaline-2,3-dione.

A useful new technique (1) that makes possible the direct measurement of synaptic currents in the central nervous system has recently evolved from the application of the patch clamp to thin slices of brain tissue. We have used this technique to record excitatory and inhibitory postsynaptic currents from synapses on Purkinje cells of the cerebellar cortex.

Cerebellar Purkinje cells receive excitatory synapses from two distinct pathways, as diagrammed in Fig. 1A (2, 3). The first of these relays information arising from sensory receptors in all parts of the body to the cerebellum, by way of mossy fibers. In mature animals, a mossy fiber makes contact with several hundred granule cells, each of which gives rise to a parallel fiber. The parallel fibers ascend through the molecular layer, bifurcate, and synapse with Purkinje cells. Each Purkinje cell receives synaptic input from as many as 80,000 parallel fibers. The path thus is from mossy fibers to granule cells to Purkinje cells. The second input, the climbing-fiber input, is strikingly different. A Purkinje cell receives input from a single climbing fiber, and transmission in this pathway is one to one. The functional significance of these two inputs is not yet certain. An interesting hypothesis holds that a climbing fiber serves as "instructor" to alter the strength of parallel fiber synapses (4, 5).

In addition to the excitatory inputs there are two inhibitory inputs, the more powerful being from basket cells. A Purkinje cell receives input from numerous basket cells.

The changes in Purkinje cell membrane potential that follow activation of the excitatory inputs have been described *in vivo* and in cerebellar slices (3). We report here observations on the postsynaptic currents elicited by (i) climbing fibers, (ii) a second excitatory pathway probably involving mossy and parallel fibers, and (iii) an inhibitory input that arises from basket cells.

METHODS

Experiments were performed on thin slices of cerebellum, taken from rats 11–17 days after birth. The cerebellum was removed and placed in ice-cold saline within 2 min of sacrificing the animal. The cerebellum was split at the midline and glued in the appropriate orientation in the cutting chamber of the slicer with cyanoacrylate glue. Slices, typically 140 μm thick, were prepared from the vermis with a vibrating slicer. For all of the experiments reported here, the slice was oriented parallel to the long axis of the folium (and the parallel fibers).

After incubation for half an hour in oxygenated saline (bubbled throughout preparation and experimentation with 95% O_2 /5% CO_2), a slice was placed in the experimental chamber and continuously superfused. A Purkinje cell was selected, using Nomarski optics with a $\times 40$ water-immersion lens, and its upper surface was cleaned by gently blowing and sucking saline from a cleaning pipette with diameter of $\approx 8 \mu\text{m}$ (1). A tight seal was formed using a Sylgard-coated patch pipette of resistance 2–3 $\text{M}\Omega$, and the membrane was then broken to achieve whole-cell configuration. Compensation for series resistance was performed using the standard features of an EPC-7 patch clamp (List Electronics, Darmstadt, F.R.G.). The cells under clamp changed very slowly and in favorable cases could be examined for many hours without noticeable change in properties.

Focal stimulation was applied from an isolated stimulus circuit by means of a second pipette with a resistance of about 1 $\text{M}\Omega$. This pipette was moved about in the vicinity of the Purkinje cell until the desired response was obtained. Stimulus intensity is reported here in terms of volts applied to this pipette. The stimulating current can be approximated by dividing this voltage by 1 $\text{M}\Omega$.

The external saline was 125 mM NaCl/2.5 mM KCl/2 mM CaCl_2 /1 mM MgCl_2 /1 mM NaH_2PO_4 /26 mM NaHCO_3 /10 mM glucose, pH 7.4. The usual internal solution was 120 mM CsCl/20 mM tetraethylammonium chloride/4 mM NaATP/2 mM MgCl_2 /10 mM EGTA/10 mM Hepes, pH 7.3. We employed Cs^+ instead of K^+ to facilitate the measurement of synaptic currents. Cs^+ blocks outward current through voltage-sensitive K^+ channels and thus simplifies interpretation

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Abbreviations: CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; GABA, γ -aminobutyrate.

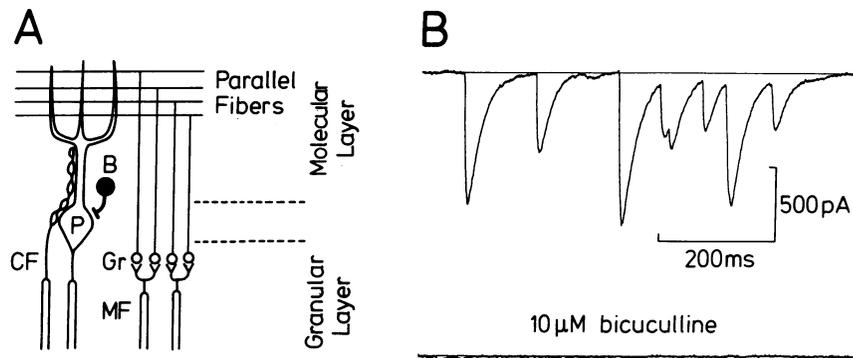


FIG. 1. (A) The major synaptic connections of a Purkinje cell (P) in the cerebellar cortex. The relevant inhibitory input is from numerous basket cells (B), which have synapses on the soma and the initial segment of the Purkinje cell axon. (Synaptic input to the basket cells is omitted for clarity.) In the mature animal, a single climbing fiber (CF) has multiple excitatory synapses on the soma and proximal dendrite of the Purkinje cell. The second excitatory input is the mossy fiber (MF) to granule cell (Gr) to Purkinje cell (P) path. A mossy fiber has synaptic contact with perhaps several hundred granule cells, and a Purkinje cell receives synaptic input from thousands of granule cells. (B) Spontaneous inward currents recorded from a Purkinje cell with high internal Cl^- (upper trace). The currents are completely eliminated by the γ -aminobutyrate type A (GABA_A) antagonist bicuculline (lower trace) and almost certainly arise from basket cell–Purkinje cell synapses. Holding potential, -60 mV.

of the records. Experiments were performed at room temperature, near 22°C .

RESULTS

All of the Purkinje cells that we examined had spontaneous currents similar to those illustrated in Fig. 1B, which ranged from 50 pA to more than 1 nA. The peak of the current was 3–6 ms after its origin, and the current decayed to half of peak amplitude ≈ 15 ms after the origin. These currents were reversibly blocked by $10 \mu\text{M}$ bicuculline (Fig. 1B, lower trace), a blocker of GABA_A receptors. Thus the currents are through GABA_A channels and almost certainly arise from spontaneous activity in the numerous basket cells that form inhibitory synapses on a Purkinje cell. The current is inward in our recording conditions (high internal Cl^- , with V_{Cl} near

0 mV), but in an unperfused cell the synapses would be inhibitory.

Postsynaptic currents elicited by the two excitatory inputs are illustrated in Fig. 2. The climbing fiber was stimulated directly over the Purkinje cell, or in the granule cell layer 50–100 μm away (Fig. 1A). The electrode was moved about until the climbing-fiber response could be elicited with the minimum stimulus intensity. An inward synaptic current elicited by the climbing fiber was easily recognized by its large amplitude (2–3.4 nA at -60 mV) and its all-or-none characteristic: with stimulus intensity near threshold, the response was either full amplitude or zero. It could be distinguished readily from an antidromic spike in the Purkinje cell axon, because the latter had a much shorter latency, had a briefer duration, and could be inactivated by holding at -30 mV.

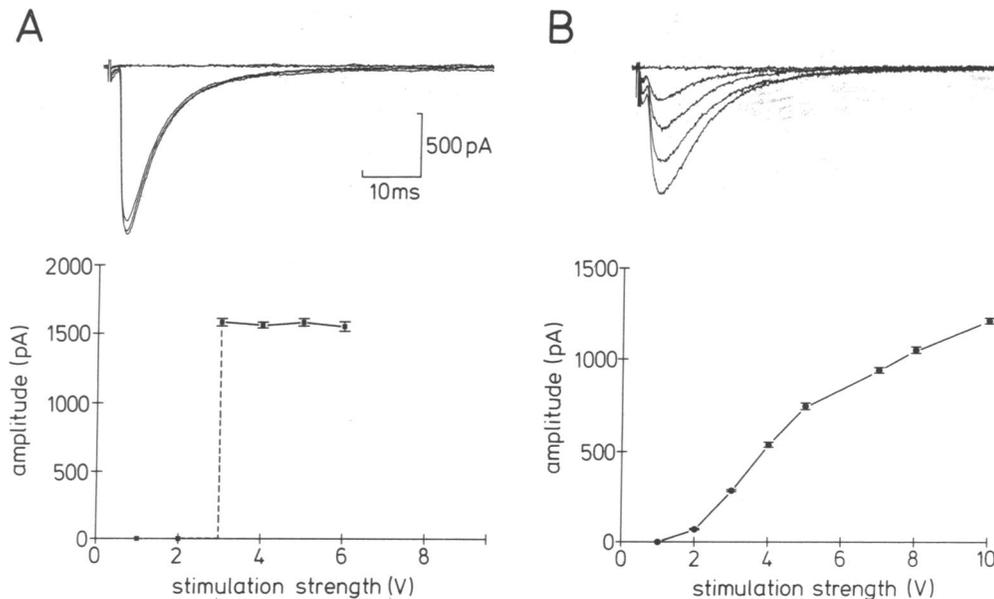


FIG. 2. Two types of excitatory postsynaptic currents in Purkinje cells. (A) (Upper) The synaptic current elicited by a climbing fiber, stimulated with intensity of 1, 2, 3, 4, and 5 V. There was no response to the stimuli of 1 and 2 V. (Lower) Synaptic current as a function of stimulus intensity. The current is all or none, depending on whether the climbing fiber fires or does not. Each point is the average (\pm SD) of the response to five successful stimuli (blanks omitted). (B) (Upper) Synaptic currents of the graded response, elicited by stimuli of 1, 3, 4, 7 and 10 V. (Lower) The synaptic current of this pathway is smoothly graded as a function of stimulus intensity. Each point represents the average value (\pm SD) from five consecutive stimuli. All recordings (A and B) were made in the presence of $10 \mu\text{M}$ bicuculline. To inactivate voltage-dependent conductances, holding potential was -30 mV, and K^+ conductance was further suppressed by Cs^+ and tetraethylammonium ions inside the cell.

The all-or-none character is evident when the amplitude of the response to successful stimuli is plotted as a function of stimulus intensity (Fig. 2A Lower). With the stimulating electrode close to the Purkinje cell ($50\ \mu\text{m}$ or less) the current began with a minimum latency of 1.9 ms (measured from the beginning of the stimulus). The average time from origin to peak was 2.0 ms, and the time for decay to half amplitude (measured from the origin of the current) was 6.8 ms at $-60\ \text{mV}$.

The second excitatory pathway could be stimulated either in the molecular layer or in the granule cell layer. The identifying characteristic of this response is its smoothly graded stimulus intensity–response relation (Fig. 2B). The temporal characteristics of the response are similar to those of the synaptic current elicited by the climbing fiber, although both the rise and fall of the current were somewhat slower. The average time to peak from onset of the current was 3.2 ms, about a millisecond slower than the climbing-fiber current. The half decay time, measured from the origin of the current, was 9.5 ms at $-60\ \text{mV}$ (CsCl inside the cell). The latency in our experiments ranged from 1.8 ms to 2.5 ms.

The excitatory synapses could also be distinguished by their response to sequential stimuli (Fig. 3). For two stimuli to the climbing fiber that were 30 ms apart, the synaptic current was reduced in the second to about 60% (*cf.* ref. 6). As the interval between the paired stimuli was increased, the second response grew slowly and had not completely recovered after 5 sec (Fig. 3A).

The graded response showed exactly the opposite behavior (Fig. 3B). For short intervals, the second response was larger than the first and fell in amplitude as the interval grew longer. For intervals of 250 ms or longer, the response to the first and second stimulus was essentially the same.

Neither the transmitter for the climbing-fiber synapse nor that for the parallel-fiber synapse has been definitely identified, although there is some evidence for the involvement of, respectively, aspartate and glutamate (3, 7). As a first step in pharmacological characterization, we applied CNQX, an agent that inhibits glutamate receptors of the quisqualate or kainate type in the micromolar or submicromolar range (8). As illustrated in Fig. 4, $2\ \mu\text{M}$ CNQX effectively eliminated

both the climbing fiber response (*cf.* ref. 9) and the graded response. This result suggests that the receptors for both pathways are glutamate receptors of quisqualate or kainate type. The size of this family of receptors and the precise properties of its individual members remain to be worked out.

DISCUSSION

Two of the three synaptic currents that we describe in this paper are clearly recognizable from the known anatomy and physiology of the cerebellar cortex. The currents that we attribute to synapses from basket cells are bicuculline-sensitive, which establishes the involvement of GABA_A receptors, and GABA is the probable transmitter at the basket cell–Purkinje cell synapse (10–12). These currents are quite variable in size, compatible with the idea that numerous basket cells synapse with a single Purkinje cell, but with different synaptic weights.

The large excitatory synaptic current, which is evoked all or none in rats that are more than a few days old, is clearly identifiable as the climbing-fiber input. In young rats we sometimes saw evidence for multiple innervation of a single Purkinje cell—i.e., the synaptic current grew in jumps with the stimulus intensity. Multiple innervation of Purkinje cells by climbing fibers has been reported in 8- to 14-day-old rats (13). This behavior was easily distinguished from the smoothly graded stimulus–intensity relation of what we call the graded response.

The minimum latency of the climbing-fiber response for stimulation close to the cell, 1.9 ms, is consistent with data in the literature for climbing-fiber excitatory postsynaptic potentials elicited by stimulation in the white matter at $36\text{--}37^\circ\text{C}$ (ref. 14, 2.1 and 5.6 ms; ref. 15, 1.8 ms), if allowance is made for the temperature ($\approx 22^\circ\text{C}$ in our experiments). Conduction velocities and synaptic delays for these pathways have not been precisely determined at either temperature.

It is interesting to speculate on the decline in the climbing fiber-evoked synaptic current when two closely spaced stimuli are applied. Although many factors, pre- and postsynaptic, may be involved, the lack of temporal summation suggests that enough transmitter is released by the first impulse

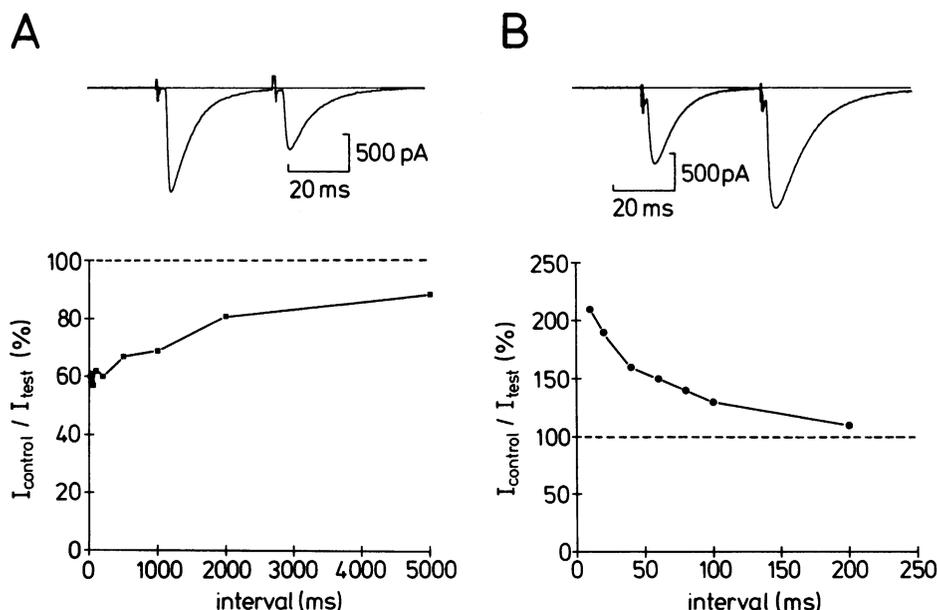


FIG. 3. The response of the two excitatory pathways to paired stimuli. (A) The synaptic current elicited by a second stimulus to the climbing fiber is depressed and recovers over the course of several seconds. (B) In contrast, the response elicited by a second stimulus to the graded path is enhanced. Each trace in A and B is the average of responses for five trials, recorded in the presence of $10\ \mu\text{M}$ bicuculline, at a holding potential of $-30\ \text{mV}$.

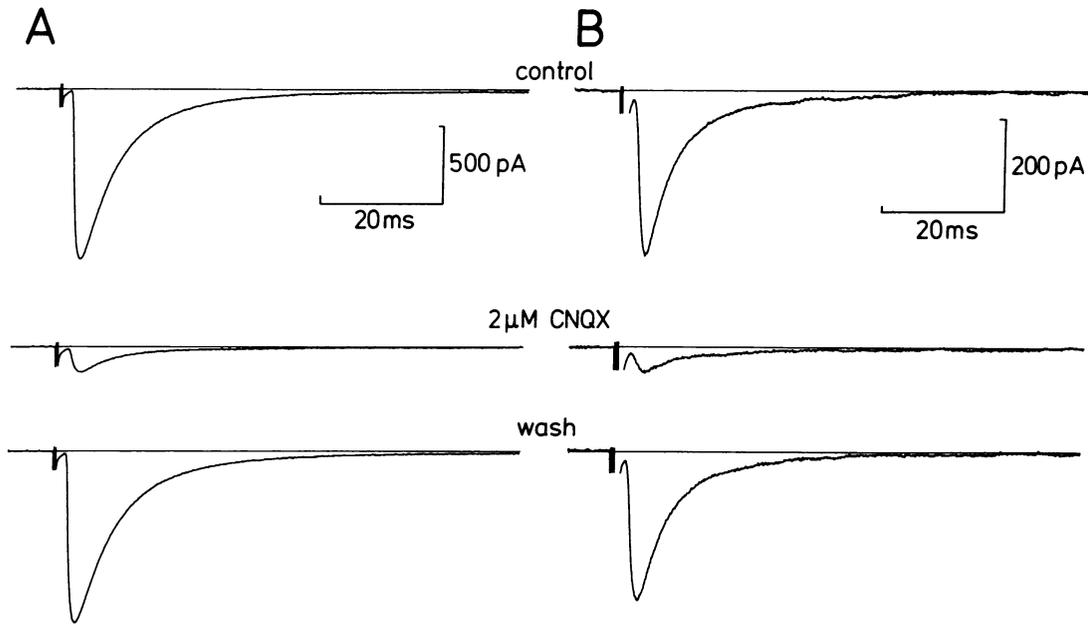


FIG. 4. Blocking of climbing-fiber (A) and graded (B) responses by the glutamate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). For both responses, traces are shown before, during, and after application of the drug. Each trace is the average of 10 consecutive stimuli, recorded in the presence of 10 μ M bicuculline. Holding potential was -30 mV (A) or -60 mV (B).

to saturate the postsynaptic receptors. The receptors themselves can be tentatively identified as non-NMDA (*N*-methyl-D-aspartate)-type glutamate receptors on the basis of their sensitivity to CNQX.

The graded response is probably associated with the mossy fiber-granule cell-Purkinje cell pathway. The response is smoothly graded as a function of stimulus intensity, as would be expected if the maximal stimulus activated a large number of mossy fibers or, alternatively, a large number of granule cells. The latency of the response suggests that both are possibilities. In some cases the latency was longer than for the climbing-fiber response, consistent with two synaptic delays and stimulation of the mossy fibers. In other cases, however, the latency was the same as for the climbing-fiber response, suggesting a monosynaptic pathway. This could be explained if there were direct stimulation of the granule cells. An alternate explanation is a direct mossy fiber-Purkinje cell synapse, as is seen in young kittens (16).

In conclusion, we have shown that the synaptic currents for three distinct synapse types on cerebellar Purkinje cells can be isolated and studied, and we have described the synaptic currents associated with these pathways. Two of the pathways are excitatory and have highly distinct properties. The transmitter for neither of the excitatory pathways is known, but the susceptibility of both to CNQX suggests that the receptors are of the quisqualate or kainate type. The third receptor is readily blocked by bicuculline and is undoubtedly a GABA_A inhibitory receptor that opens a Cl⁻ channel.

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