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Sequential stimulation of rat and guinea pig cerebellar granular cells in vitro leads to increasing population activity in parallel fibers

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Abstract

Sequential stimulation of the granular layer of the cerebellar cortex in vitro using 11 linearly aligned stimulating electrodes leads to massive population activity in the parallel fiber system and to spike activity in Purkinje cells (Heck, D., Neurosci. Lett., 157 (1993) 95–98; Heck, D., Naturwissenschaften, 82 (1995) 201–2030). The induced parallel fiber activity, however, might have been a result of direct stimulation of parallel fibers themselves and not of stimulation of granular cells or their ascending axons. We report here that using sequential 'moving' stimuli and varying the distance covered by the 'movement', parallel fiber population spike amplitude increases with distance and saturates for distances longer than 1.0 mm. This effect cannot be explained if parallel fibers are directly stimulated, but requires stimulation of the granular cells or their ascending axons. We conclude that the population spike activity and Purkinje cell responses induced by sequential stimulation of the granular layer of the cerebellar cortex slices in this and earlier experiments consists of orthodromic parallel fiber spikes.

Keywords: Cerebellum; Parallel fiber; Spatio-temporal activity; Dynamic input; Movement detection; Synchronization

In the cortex of the cerebellum a large number of unmyelinated fibers (the parallel fibers) reach from each point of the cortex a few millimeters to the left and to the right. They originate from a T-shaped bifurcation of the ascending axon of granular cells. The excitatory granular cells [7] relay input from mossy fibers to Purkinje cells. Each Purkinje cell receives excitatory input from about 200 000 parallel fibers [14]. Purkinje cells have a characteristically flat dendritic tree, which extends (in rats) about 200 μm by 200 μm in the sagittal plane but is only about 10 μm thick. The parallel fibers intersect the plane of Purkinje cell dendritic trees at right angles. With a total length of roughly 5 mm in rats [15], each parallel fiber contacts several Purkinje cells, whose somata are about 30 μm apart. Conduction velocity of parallel fibers is about 0.3–0.5 m/s [6,9].

It has been shown in theoretical [2,3] and experimental work [9,11,12] that the cerebellar cortex converts sequential granular cell activity into synchronized volleys of parallel fiber spikes, which strongly excite Purkinje cells. As a sequential input 'jumps' from one granular cell to the next it 'moves' with a certain velocity depending on the time intervals between 'jumps'. Spikes in the parallel fiber system will be perfectly aligned when the sequence of inputs 'moves' exactly at the conductance velocity in parallel fibers [11] (Fig. 1).

We show here that the amplitude of the parallel fiber population spike induced by 'moving' input strongly depends on the distance covered by the 'movement'. Population spike amplitude increases with increasing distance and, due to limited length of parallel fibers, saturates for distances longer than 1 mm.

A detailed description of the experimental procedure has been given elsewhere [9–11]. In short, acute slices (400 μm thick) were prepared from the vermis of the cerebellar cortex of 3–4-week-old rats (Sprague–Dawley) ($n = 3$) and Guinea pigs (200–400 g) ($n = 2$). Experiments were per-

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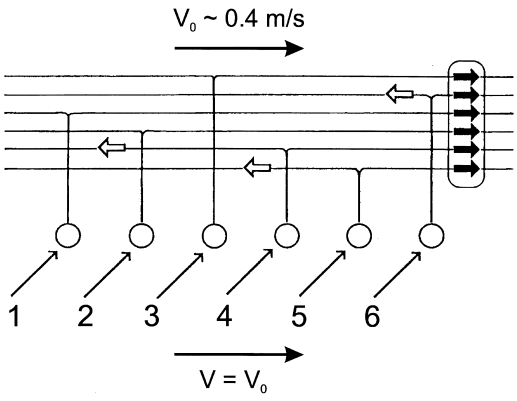

Fig. 1. Schematic drawing of cerebellar granular cells (small circles) with their axons, the parallel fibers and a Purkinje cell (rectangle). Each granular cell produces an action potential in response to a sequence of inputs which excites the leftmost cell (1) first and 'moves' to the right (2, 3, 4, 5, 6) at a virtual velocity identical to the conductance velocity in parallel fibers. At the point of bifurcation an action potential is conducted down each branch of the parallel fiber (left branch white arrows, right branch black arrows). As a consequence, the action potentials travelling in the direction of the sequential input line up. The spatially aligned spikes produce a highly synchronized input to cells with a flat dendritic tree (Purkinje, stellate and basket cells).

formed in two different species to test for species specific differences, especially with respect to differences in developmental status of the nervous system. Guinea pigs are born with an almost fully developed nervous system whereas rat brains are born immature and develop over a period of several weeks after birth [5]. Slices were cut at an orientation parallel to the long axis of the folia so that parallel fibers stayed intact. The slices were preincubated for at least 60 min in an artificial cerebrospinal fluid (ACSF) containing for the rat (mM): NaCl (124), KCl (5), KH_2PO_4 (1,15), $MgSO_4 \cdot 7H_2O$ (1.15), $NaHCO_3$ (25), $CaCl_2 \cdot 2H_2O$ (2.0) and D-glucose (10) and for the guinea pig (mM): NaCl (124), KCl (5), NaH_2PO_4 (1,15), $MgSO_4 \cdot 7H_2O$ (1.3), $NaHCO_3$ (26), $CaCl_2 \cdot 2H_2O$ (2.5) and D-glucose (10) continuously gassed with carbogen (95% O_2, 5% CO_2). For the experiments, slices were transferred to a recording chamber where they lay fully submerged in ACSF at a temperature of 33°C.

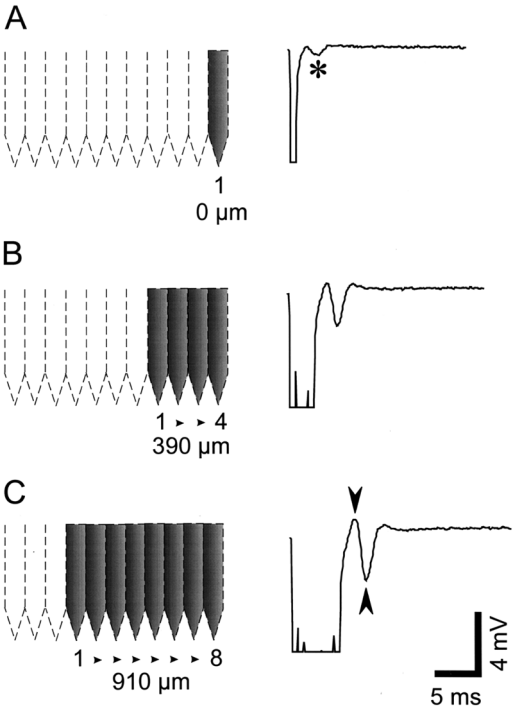

Fig. 2. Schematic drawing of the array of 11 stimulating electrodes (left) and voltage traces (right) recorded in rat cerebellar slices in response to stimulation. Voltage traces are single trial recordings. An array of 11 stimulating electrodes was used to produce 'moving' stimuli covering 11 different distances. Out of the 11 electrodes (indicated by dotted lines) some or all were used to produce a 'moving' stimulus (filled electrodes) resulting in the parallel fiber response shown on the right. Three representative examples are shown. Each voltage trace starts with an artifact (downward deflection) caused by the electrical stimulus. (A) Stimulation with only one electrode corresponds to a distance of 0 μm and produces a visible but weak population spike. (B,C) Two examples of population spike responses to a stimulus 'moving' over a distance of 390 μm or 910 μm. For further analysis, the magnitude of the population response was measured as the difference between the maximum and minimum in the voltage trace indicated by the arrowheads in (C).

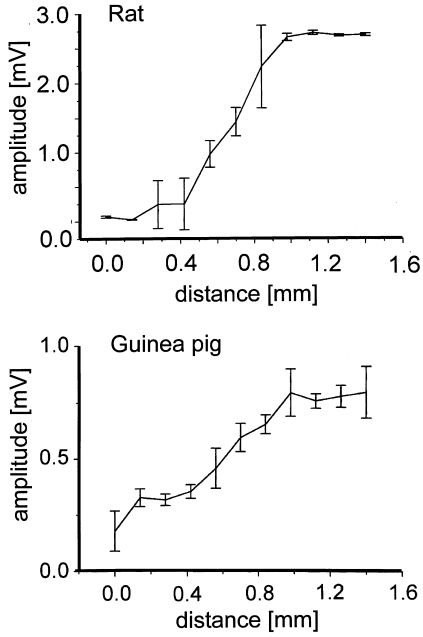

Fig. 3. Amplitude of population spike in response to a 'moving' stimulus recorded in rat (top) and guinea pig (bottom) plotted against the distance covered by the 'movement'. Population spike amplitude increases with distance and saturates for distances longer than about 1 mm in both species. Error bars represent standard deviation.

For the generation of sequential stimuli, an array of 11 linearly aligned stimulating electrodes was custom made [10]. Tip distance between electrodes (epoxy-insulated tungsten wire, AM-Systems, USA) was 130 μm. Short current pulses (50 μs) at a current strength between 200 and 500 μA were used for sequential electrical stimulation.

Using ordered sequences (e.g. electrode 1, then 2, 3, 4,…,11) the stimulus 'moved' along the array from the first electrode to the last. Altering the interval duration between stimuli changed the velocity of the 'movement'. For all experiments reported here, the velocity was set to match the conductance velocity of parallel fibers (0.35–0.4 m/s) in order to get the largest possible population spike response in the parallel fiber system [9].

The array of stimulating electrodes was placed in the granular layer of the vermal part of the slice. Stimulus current strength was adjusted so that a moving input covering the longest possible distance (11 electrodes = 1300 μm) elicited a reproducible and strong population spike of at least 0.5 mV amplitude. Stimulus current was the same for each of the 11 electrodes during one experiment.

Extracellular recordings were performed using glass micropipettes filled with 3 M NaCl (4 and 9 MΩ). The recording electrode was positioned somewhere in the middle between the pial surface and the Purkinje cell layer of the molecular layer. The distance between stimulating electrodes and recording electrode was 500–600 μm in all experiments.

Using all 11 electrodes of the array, a 'moving' stimulus was produced that traveled over a distance of 1.3 mm (10 × 130 μm tip distance). In all experiments the sequential stimulus 'moved' towards the recording site and ended at the last electrode, i.e., the one closest to the recording site (no. 11 in Fig. 4).

We varied the distance covered by the 'movement' by adjusting the number of electrodes used to produce the sequence. A 'movement' of the input over a distance of 130 μm was produced by using two neighboring electrodes, a distance of 260 μm using three and so on. Stimulus current was the same for all 11 electrodes.

Stimulating the cerebellar granular layer with a 'moving' sequential input generated a strong population spike response in the parallel fiber system. Fig. 2A,B show signals recorded in response to three different distances: 0 μm (one electrode), 390 μm (four electrodes) and 910 μm (eight electrodes).

The parallel fiber population spike (asterisk in Fig. 2A) clearly increases in size with increasing distance. Population spike amplitude was measured as the difference between the two points in the voltage trace marked by the arrow heads in Fig. 2C, and responses were plotted as a function of distance covered by the 'moving' stimulus. Fig. 3 shows representative results obtained in cerebellar slices from rat (n = 3) and guinea pig (n = 2). In both species and all experiments the

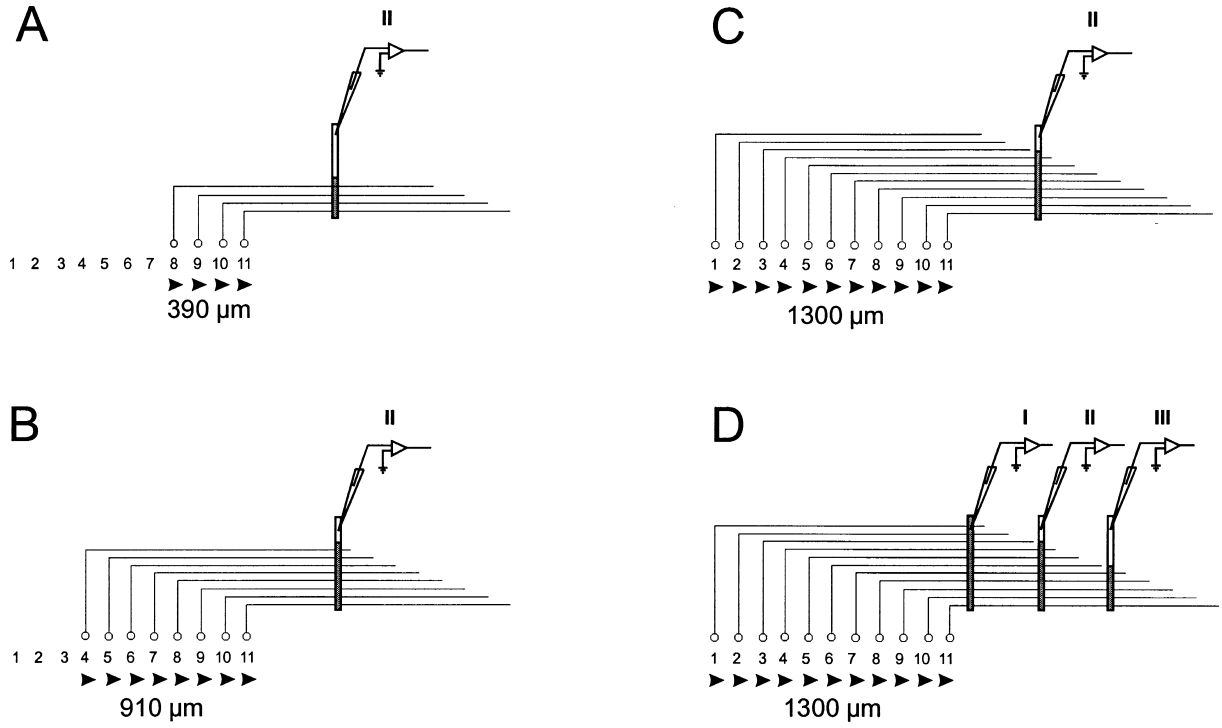

Fig. 4. Schematic illustration of the accumulation of population activity in the parallel fiber system depending on the distance covered by the 'moving' input and on the distance between recording site and the array of stimulating electrodes. For simplicity of the scheme, each electrode stimulates a single granular cell. Only one branch of each parallel fiber is drawn. The vertical rectangles represent the area from which the extracellular electrodes depicted above each rectangle collect neuronal activity. Dark shading corresponds to amplitude of population spike. (A–C) Saturation of the population spike signal. Electrode II records a larger population signal when the distance covered increases from 390 μm (A) to 910 μm (B) but further increasing the distance to more than 910 μm (C) does not change the population spike amplitude because the fibers 1, 2, and 3 never reach the recording site. (D) Generating a 'moving' input using 11 electrodes excites all 11 parallel fibers. Because parallel fibers are only a few millimeters long, only electrode I picks up the signals from all active parallel fibers. Electrodes II and III pick up less activity because they are further away from the array of stimulating electrodes.

signal increased with the distance and saturated when the distance covered reached 910 μm (Fig. 3). These data are in accordance with the assumption that granular cells or their axons are stimulated orthodromically with the method used here and that parallel fibers are not stimulated directly. If the latter was true response amplitudes would be independent of distance covered by the movement.

Fig. 4A–C shows schematically how a sequential input stimulates several parallel fibers and how more parallel fibers become active for longer distances covered by the 'movement' of the input. Parallel fiber population spike amplitude reaches saturation when the distance covered by the 'movement' plus the distance between recording electrode and the array of stimulating electrodes equals the length of parallel fibers (cf. Fig. 4B,C). From these indirect data the length of each branch of a parallel fiber in the slice would estimate to 1.6 mm. With more direct anatomical methods, however, a length of 2.1–2.35 mm for each branch of the rat parallel fiber has been found [15]. A possible reason for the difference is that parallel fibers in the slice might be partially cut off due to a non-perfect sagittal angle. Longer fibers might have been present in the slice but if they were only few, their population signal might have been too small to be detected.

Earlier experiments have shown that sequential stimulation of the granular layer of a cerebellar cortical slice led to suprathreshold activation of intracellularly recorded Purkinje cells [11]. In those experiments, however, direct stimulation of parallel fibers and thus antidromic activation of ascending axons of granular cells could not be excluded. In vivo experiments led to the notion that pure parallel fiber input cannot drive Purkinje cells to suprathreshold membrane potentials. Instead, synaptic inputs from the ascending axons of granular cells were thought to be solely responsible for rate increases measured in response to mossy fiber input [1,13]. The results presented here confirm that population spike activity induced by sequential stimulation of the granular layer of cerebellar cortical slices consists of orthodromic parallel fiber spikes without antidromic activation of ascending granular cell axons. In the ongoing discussion on the effectiveness of parallel fibers in exciting Purkinje cells [8] these findings argue in favor of the view that Purkinje cells can in fact be driven by pure parallel fiber input because the same stimulus paradigm described here induced spike activity in intracellularly recorded Purkinje cells [11]. Furthermore, the presented results add to and confirm earlier reports of the cerebellum responding specifically to 'moving' input [9,11], a mechanism that might play an important role in motor control [4].

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