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Cross-correlations between pairs of neurons in cerebellar cortex *in vivo*

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ABSTRACT

In the present paper we apply a new neurophysiological technique to make single-electrode, dual loose-patch recordings from pairs of neuronal elements in the cerebellar cortex *in vivo*. The analyzed cell pairs consisted of an inhibitory molecular layer interneuron and a Purkinje cell (PC) or a Golgi cell and a granule cell, respectively. To detect the magnitude of the unitary inhibitory synaptic inputs we used histograms of the spike activity of the target cell, triggered by the spikes of the inhibitory cell. Using this analysis, we found that single interneurons had no detectable effect on PC firing, which could be explained by an expected very low synaptic weight of individual interneuron–PC connections. However, interneurons did have a weak delaying effect on the overall series of interspike intervals of PCs. Due to the very high number of inhibitory synapses on each PC, a concerted activation of the interneurons could still achieve potent PC inhibition as previously shown. In contrast, in the histograms of the Golgi cell–granule cell pairs, we found a weak inhibitory effect on the granule cell but only at the time period defined as the temporal domain of the slow IPSP previously described for this connection. Surprisingly, the average granule cell firing frequency sampled at one second was strongly modulated with a negative correlation to the overall firing level of the Golgi cell when the latter was modified through current injection via the patch pipette. These findings are compatible with that tonic inhibition is the dominant form of Golgi cell–granule cell inhibition in the adult cerebellum *in vivo*.

1. Introduction

Analysis of neuron-to-neuron connections *in vivo* is an important approach since it provides information on the potency of single synaptic inputs on a target neuron, which in turn is crucial for our understanding of how the neuronal microcircuitry could work. For the molecular layer interneuron input to Purkinje cells (PCs), it is estimated that there are several thousand inhibitory interneuron synapses on each PC (Palay & Chan-Palay, 1974). *In vivo*, these interneurons have a spontaneous activity exceeding 10 Hz (Jörntell, Bengtsson, Schonewille, & De Zeeuw, 2010), and are firing at several hundreds of Hz during appropriate input (Jörntell & Ekerot, 2002, 2003). Since the summed activity of the interneurons would translate to at least 10 kHz of inhibitory synaptic inputs in the PC, it seems unlikely that the individual interneuron-to-PC synapses have any substantial weight. For example, if each synapse generated an average inhibitory postsynaptic current (IPSC) of 10 pA for 10 ms (Kano, Fukunaga, & Konnerth, 1996; Kondo & Marty, 1998), then even at rest the PC would be constantly depressed by a current of several thousand pA, which would be comparable only to the magnitude of the current generated by the climbing fiber synapse (Silver, Momiyama, & Cull-Candy, 1998). In a study using dual patch clamp recordings *in vitro*, the interneuron-to-PC IPSP was indeed found to be quite weak (in the range 0.05–0.61 mV) (Häusser & Clark, 1997). For many reasons, these amplitudes may be expected to be much lower *in vivo* (see the discussion).

How can we find connected pairs of interneurons and PCs? In the cerebellum, the classical concept is that interneuronal inhibition is organized according to the so-called parallel fiber beams where the activity in one beam triggers inhibition in neurons located "off-beam" (Eccles, Ito, & Szentágothai, 1967), also illustrated in more recent studies (Dizon & Khodakhah, 2011; Gao, Chen, Reinert, & Ebner, 2006). However, intracellular recordings both *in vivo* and *in vitro* have shown that electrical or optical activation of a beam of parallel fibers also evokes powerful inhibition in neurons located on-beam, although this is also mixed with on-beam excitation (Dizon & Khodakhah, 2011; Jörntell et al., 2010; Jörntell & Ekerot, 2003; Mittmann & Häusser, 2007). Anatomical analysis of interneurons, either in dedicated studies (Sultan & Bower, 1998) or by examples of morphologically recovered interneurons published in papers with other main purposes (Christie & Jahr, 2008; Hull & Regehr, 2012; Pugh & Jahr, 2011), show that the branching of the axon and the number of synaptic boutons have the highest densities nearby the soma. Paired recordings of adjacent basket cells and Purkinje cells also directly demonstrated that on-beam inhibition exists between these two types of neurons when they are both located on-beam (Hull & Regehr, 2012). These anatomical

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and physiological findings indicate that the inhibitory interneurons have strong on-beam effects and that interneurons and PCs located adjacent to each other should have a good probability of being synaptically connected.

In contrast to the interneuron-to-PC synaptic communication, there exist no good indications of how many Golgi cells may innervate each granule cell. The closest estimate seems to stem from the ultrastructural study of the glomerulus made by (Jakab & Hamori, 1988). In this paper, the number of Golgi cell axons per glomerulus is estimated as 1–2. For a granule cell with four dendrites (Cathala, Brickley, Cull-Candy, & Farrant, 2003), this would amount to a total of 4–8 Golgi cell inputs per granule cell. The major source of uncertainty is the estimate of Golgi cell inputs to the single glomerulus since Jakab and Hamori (1988) investigated only two glomeruli. However, it is clear that the granule cell receives synaptic inputs from very few Golgi cells.

In contrast to the fast, classical inhibitory response in interneuron-to-Purkinje cell synapses, the Golgi cell to granule cell inhibition has a prominent tonic inhibitory component (Brickley, Cull-Candy, & Farrant, 1996; Chadderton, Margrie, & Häusser, 2004; Duguid, Branco, London, Chadderton, & Häusser, 2012; Jörntell & Ekerot, 2006; Wall & Usowicz, 1997). For this connection, there exists little doubt that vicinity means connectivity: the Golgi cell issues an axon that branches profusely to cover the volume around its soma with densely packed synaptic terminals (Eccles et al., 1967; Holtzman, Rajapaksa, Mostofi, & Edgley, 2006).

In the present paper, we draw on the advantage of a recently described technique for loose-patch dual extracellular recordings (Bengtsson & Jörntell, 2009) to describe the cross-correlations between such neuron pairs in the non-anesthetized decerebrated cat preparation. For this purpose, the choice of preparation is important as most known anesthetics potentiate inhibition (Bengtsson & Jörntell, 2007). We find that single interneurons have only a little effect on the PC simple spike firing, probably due to the shunting of small individual IPSPs by a massive background of other inputs *in vivo*. In contrast, our data on the Golgi cell to granule cell connection indicates a comparably strong inhibitory effect partly at the time window of slow IPSPs *in vivo* (Jörntell & Ekerot, 2006) but in particular across longer time periods (second-by-second).

2. Materials and methods

2.1. Preparation

Adult cats were prepared as previously described (Jörntell & Ekerot, 2002, 2003). Briefly, following an initial anesthesia with propofol (Diprivan® Zeneca Ltd, Macclesfield Cheshire, UK), the animals were decerebrated at the intercollicular level and the anesthesia was discontinued. The animals were artificially ventilated and the end-expiratory CO_2, blood pressure and rectal temperature were continuously monitored and maintained within physiological limits. Mounting in a stereotaxic frame, drainage of cerebrospinal fluid, pneumothorax and clamping the spinal processes of a few cervical and lumbar vertebral bodies served to increase the mechanical stability of the preparation. Our EEG recordings were characterized by a background of periodic 1–4 Hz oscillatory activity, periodically interrupted by large-amplitude 7–14 Hz spindle oscillations lasting for 0.5 s or more. These forms of EEG activities are normally associated with deep stages of sleep (Niedermayer & Lopes da Silva, 1993). The pattern of EEG activity and the blood pressure remained stable, also on noxious stimulation, throughout the experiments.

2.2. Recordings and stimulation

The initial delineation of the forelimb area of the C3 zone in the cerebellar anterior lobe and the continuous monitoring of the general condition in the sensitive mossy fiber-to-granule cell-to-parallel fiber pathway were performed as described previously (Ekerot & Jörntell, 2001; Jörntell & Ekerot, 2002). *In vivo*patch clamp recordings were made in the lower molecular layer, the Purkinje cell layer and the granule cell layer in the tip of the folia accessible from the surface of the cerebellum. We used patch pipettes pulled to 6–14 MΩ (potassium-gluconate-based internal solution) on a Sutter micropipette puller (P-97, Sutter Instruments Co., USA). Loose patch recordings were obtained on a routine basis as a result of failed attempts to obtain Giga-Ohm seals. The present analysis was confined to the rare cases in which two distinct unitary spikes could be detected in the same recording. This method is described in further detail in (Bengtsson & Jörntell, 2009).

2.3. Analysis

Using home-made software and the Data Translation 3010 A/D board, all recordings were continuously sampled and digitized at 100 kHz. Off-line analysis of spike times was made in other custom-made software. For estimation of cross-correlations, one of the unitary two spikes recorded (in this paper the spike identified as an interneuron or a Golgi cell) was set as the trigger ('primary' spike). The software then identified the relative times of the other unitary spike ('secondary' spike) over a 2 s time window that straddled the time point of the trigger spike. In cases where the two spikes coincided or nearly coincided, the time was determined by close inspection of the trace. Typically, when spikes partly overlapped in time, they generated a voltage trace that could be accounted for by simple addition of the waveforms of the two spikes (Bengtsson & Jörntell, 2009) and the start point of a spike was still easy to identify by a distinct break in the normal time course of the other spike. The procedure was repeated for every primary spike encountered and a frequency distribution histogram of the spike times of the secondary spike was created. For each cell pair analyzed, the number of primary spikes was more than 1000 (with one exception for an Int-PC pair with only 400 primary spikes), in some cases more than 10,000. From these histograms, we subtracted the baseline activity of the secondary spike and calculated the net, average change in this spike's activity for the time windows 1–25 and 26–200 ms, respectively, after the primary spike. The time windows were chosen to fit the expected time courses of fast (Häusser & Clark, 1997) and slow IPSPs (Jörntell & Ekerot, 2006), respectively.

All experiments were approved in advance by the local Swedish Animal Ethics Committee.

3. Results

Using the dual loose-cell patch technique (Bengtsson & Jörntell, 2009), we recorded from 23 pairs of molecular layer interneurons and PCs as well as 17 pairs of Golgi cells and granule cells. Of these recordings, sufficient amount of data was obtained only from 8 pairs of interneurons and PCs, and from 6 pairs of Golgi cells and granule cells.

3.1. Interneuron and PC pairs

Dual recordings of interneurons and PCs were obtained in the lowermost part of the molecular layer and in the PC layer. Due to the recording depth most of the recorded interneurons are likely to have been basket cells or basket cell axons (Southan & Robertson, 1998). Fig. 1(A) illustrates an example of a dual interneuron and PC recording. In this case, the spike amplitude of the interneuron was substantially smaller than that of the PC. Across the material, the ratio between the spike amplitudes of the PC and the interneuron could vary between 1:10 to 10:1, approximately. The