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ORIGINAL PAPER

Golgi Cell Activity During Eyeblink Conditioning in Decerebrate Ferrets

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Abstract Golgi cells have a central position in the cerebellar cortical network and are indirectly connected to Purkinje cells, which are important for the acquisition of learned responses in classical conditioning. In order to clarify the role of Golgi cells in classical conditioning, we made extracellular Golgi cell recordings during different stages of conditioning, using four different conditional stimuli. Our results show that forelimb and superior colliculus stimulation, but not mossy fiber stimulation, evokes a short latency increase in Golgi cell firing. These results suggest that Golgi cells are involved in modulating input to the cerebellar cortex. There were however no differences in Golgi cell activity between naïve and trained animals, which suggests that Golgi cells are not intimately involved in the plastic changes that occur during classical conditioning. The absence of long latency effects of the conditional stimulus also questions whether Golgi cells contribute to the generation of a temporal code in the granule cells.

Keywords Classical conditioning · Cerebellum · Golgi cells · Timing · In vivo electrophysiology

Main Text

In classical eyeblink conditioning, repeated presentations of a neutral conditional stimulus (CS), followed by a reflex-eliciting unconditional stimulus (US), result in the gradual acquisition of a conditioned blink response (CR). Acquisition of CRs is dependent on the cerebellar cortex [1]. During conditioning, Purkinje cells develop an adaptively timed pause response, a "Purkinje cell CR" (Fig. 1C), thought to be the neural foundation of the conditioned eyeblink [2]. Purkinje cells receive information about the CS from the mossy/parallel fibers and information about the US via climbing fibers, originating in the inferior olive. Both of these input pathways form synapses with other cells in the cerebellum, raising the possibility that these are involved in the learning process.

Golgi cells are richly interconnected interneurons occupying a central position in the granular layer of the cerebellar cortex. They receive information from the periphery, via mossy and parallel fibers, and they communicate with other cortical interneurons, including other Golgi cells, via gap junctions [3, 4]. Though it was initially thought that Golgi cells also receive input from climbing fiber collaterals [4], recent evidence indicates that there are few, if any, such connections [5]. Golgi cells are the sole source of inhibition onto granule cells, the origin of parallel fibers. Studies of transgenic mice, in which Golgi cells have been eliminated, as well as extracellular Golgi cell recordings, demonstrate their involvement in motor coordination and locomotion [6, 7]. A widespread view is that their interaction with granule cells (Fig. 1A,B) generates a temporal code that can outlast a sensory signal. Such a code would enable Purkinje cells to initiate actions, with a delay, because different sets of granule cells would be active at different times [8, 9]. Given their extensive connectivity and proposed role in tasks requiring motor coordination, Golgi cells might plausibly be involved in the acquisition of conditioned eyeblinks.

To investigate their contribution to eyeblink conditioning, we recorded the extracellular activity of 21 putative Golgi cells, in 21 male decerebrate ferrets (study approved by local

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ethical committee). The experimental setup is illustrated in Fig. 1A. For full details on surgical procedures, see [2]. Golgi cell identification was based on recording depth, absence of complex spikes, average instantaneous frequency (Fig. 1E), and short latency responses to climbing fiber stimulation (Fig. 2B) [10]. To enable comparisons, we also calculated measures used by Ruigrok et al. [11] to categorize different cell types. With the exception of the average instantaneous frequency, which appears to depend on the setup, our observations were similar to those presented in prior studies [11, 12] (Table 1).

All Golgi cells were found in eyeblink controlling areas of the cerebellar cortex, as confirmed by large field potentials following periorbital stimulation. Recordings were made at various stages during longer periods of training, in which a 300-ms CS was paired with a co-terminating US. The CS consisted of electrical stimulation (at 50 Hz) delivered to the forelimb (n=12, I=0.5–2 mA), mossy fibers (n=4, I=100–120 μA), superior colliculus (n=4, I=150–200 μA), or pontine nuclei (n=1, I=150 μA). The US consisted of two sets of five 500-Hz climbing fiber stimulus pulses (n=19, I=100–300 μA) or three 50-Hz periorbital stimulus pulses (n=2, I=3 mA). The intertrial interval was 15±1 s, and the interstimulus interval was 300 ms.

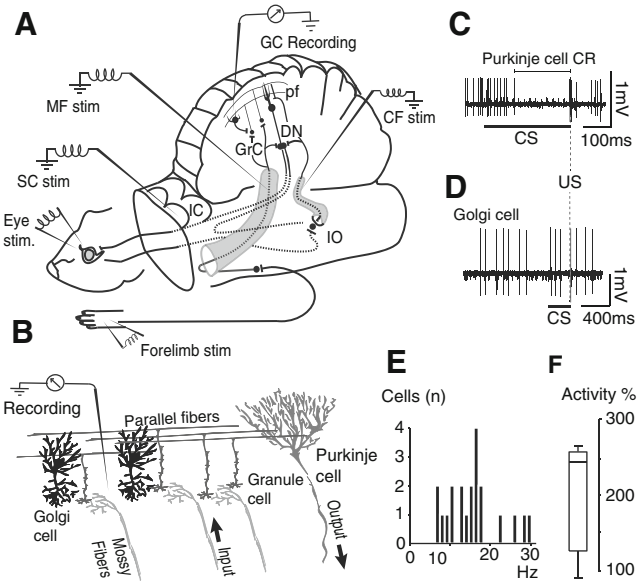

Fig. 1 Methods, circuitry, and Golgi cell activity. **a** Cerebellar and extracerebellar pathways, cell types, and stimulation sites. **b** Local cerebellar circuitry and Golgi cell connectivity. **c** Raw data sweep showing a Purkinje cell CR. **d** Raw data sweep showing Golgi cell activity. **e** Histogram showing the distribution of mean instantaneous Golgi cell frequency in all cells (n=21). **f** Boxplot illustrating the distribution of Golgi cell activity 5–25 ms into the CS period, relative to background firing (n=21)

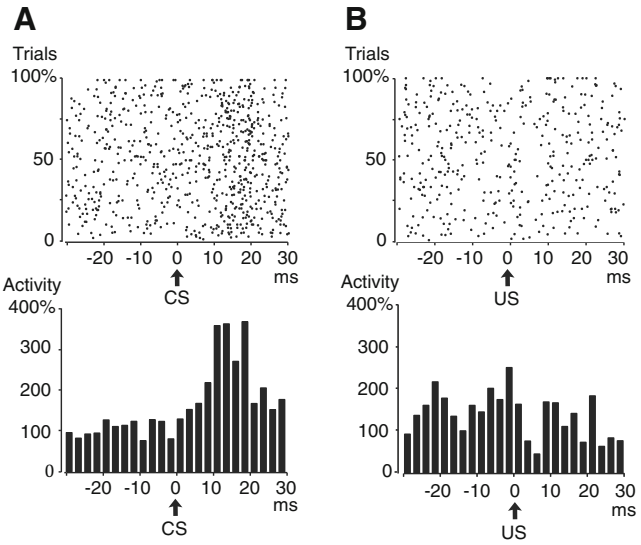

Fig. 2 Histogram (normalized firing rate, bin size=2 ms) and raster plots (all spikes from all sessions), illustrating Golgi cell response to the CS and US. **a** The conditional stimulus causes a short latency increase in Golgi cell firing. **b** The unconditional stimulus causes a short latency inhibition of Golgi cell activity

To assess whether Golgi cells changed their response to the CS as a result of training, we compared cells from trained versus naïve animals. An animal was categorized as "trained" if it had received more than 3 h of training (720 trials of paired CS–US stimuli) and had Purkinje cells exhibiting CRs [2] (n=8). Animals that had received less than 1 h of training were categorized as naïve (n=7). The six remaining animals that had received more than 1 h of training but did not exhibit CRs were excluded from this analysis. We also performed a separate analysis of Golgi cell responses to each of the different types of CSs used (see above), independent on the amount of training that cell had received. All spike sorting was done in Spike2, version 7 (Cambridge Electronics Design). Subsequent data analysis was made using custom Matlab scripts (Mathworks).

Table 1 Descriptive statistics of Golgi cell activity compared to prior studies [11, 12]. For calculation details, see [11]

	Rasmussen et al.	Ruigrok et al.	Jirenhed et al.
Cvlog	0.08±0.03	0.11±0.02	0.10±0.07
Avg.Freq	16.2±6.40	5.93±3.10	19.1±8.5
CV2	0.30±0.09	0.54±0.13	0.35±0.18
ISIperc05	0.04±0.02	0.08±0.03	0.04±0.02
ISImed	0.06±0.02	0.17±0.09	0.06±0.03

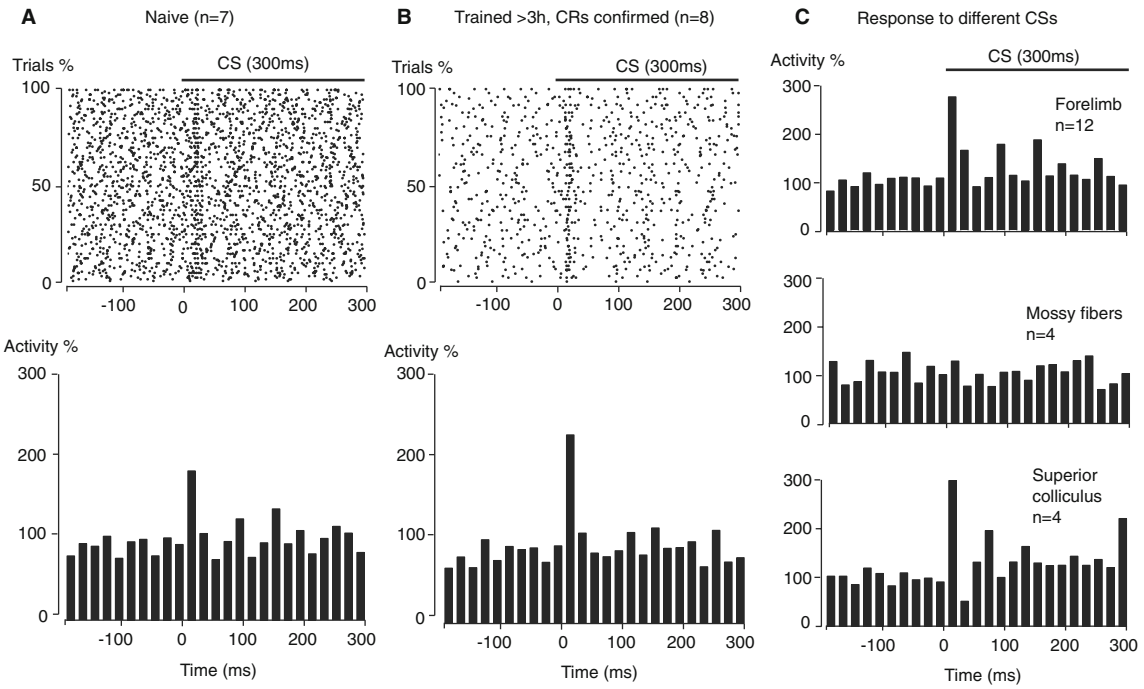

Fig. 3 Histograms of firing rates normalized within each cell to the mean background firing rate (bin size=20 ms) and raster plots (all spikes from all sessions, *y*-axis for individual cells adjusted to fit all trials in the window), illustrating Golgi cell activity from 200 ms before CS onset to the end of the CS. **a** Cells in animals trained for 1 h or less. **b** Cells in animals trained 3 h or more with confirmed Purkinje cell CRs in nearby Purkinje cells. **c** Golgi cell responses to the various types of CSs used (pontine stimulus session not shown)

With the exception of mossy fiber stimulation, all CSs elicited a short latency, brief increase in Golgi cell firing. As illustrated in Fig. 1F, the median Golgi cell firing rate 5–25 ms into the CS period was 243 % of background firing. The increase began about 10 ms after CS onset, lasting for about 15 ms (Fig. 2A). A Mann–Whitney–Wilcoxon test showed that there was no difference between naïve ($n=7$) and trained ($n=8$) animals, in Golgi cell activity 5–25 ms into the CS ($U=57$, $p=0.46$, two-tailed) (Fig. 3A,B). A second test revealed that there was no significant difference between trained and naïve animals in Golgi cell activity over the entire 300-ms CS period ($U=59$, $p=0.61$, two-tailed). In short, we did not find any evidence that Golgi cell activity differs depending on whether the animal had been trained or not. The absence of any training-induced changes, together with the previous finding that Golgi cell activity cannot account for the changes in Purkinje cell activity following saccadic adaptations [13], suggests that Golgi cells are not directly involved in the modulation of Purkinje cell activity during motor learning [2]. This conclusion may appear to contradict the finding that paired peripheral and climbing fiber stimulation can produce plastic changes in Golgi cell firing [14]. However, in their study, Xu and Edgley only saw changes following simultaneous stimulation of the two afferents. When an interval of either 750 or 1,050 ms between the peripheral and climbing fiber stimulus was introduced, no plastic changes were observed [14]. Whether these Golgi cell response patterns are still compatible with the hypothesis that interactions between Granule cells and Golgi cells generate a temporal code [8, 9] is a question that should be addressed in future studies.

One surprising observation was that in both naïve and trained animals, mossy fiber stimulation did not, like the other CSs, produce any changes in Golgi cell activity (Fig. 3C). We are unable explain this discrepant response pattern although one possibility is that the other CSs (forelimb, SC, and pontine stimulation) activated alternative afferent pathways, leading to increased Golgi cell firing. It should be noted, however, that these results are consistent with earlier reports that Golgi cells respond in complex ways to peripheral stimulation [13, 15].

In summary, Golgi cells typically respond to the CS with a short latency, brief excitation. However, the observation that Golgi cell activity in trained animals did not differ from activity in naïve animals suggests that Golgi cells are not directly involved in the plastic changes that occur during conditioning.

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Conflict of Interest None

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