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RESEARCH NOTE

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Nociceptive visceral stimulation modulates the activity of cerebellar Purkinje cells

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Abstract The cerebellum is a system with various input and output functions that influence motor, sensory, cognitive, and other processes. In a previous study, we showed that cerebellar cortical stimulation increases spinal neuronal responses to visceral noxious stimulation by colorectal distension (CRD). However, the neuronal network underlying the cerebellar modulation of nociceptive phenomena is largely unknown. Purkinje cells of the cerebellar cortex receive ascending and descending inputs and exert a major inhibitory control over neurons in the underlying cerebellar nuclei that constitute the cerebellar output. Therefore, in this study, we tested the effect of CRD and other somatic stimuli on the firing rate of Purkinje cells using in vivo extracellular recording techniques. The results suggest that Purkinje cells respond to nociceptive visceral and somatic stimulation in the form of early and delayed changes in activity. Based on these and previous findings, we propose a negative feedback circuitry involving the cerebellum for the modulation of peripheral nociceptive events.

Keywords Nociception · Visceral · Colorectal distension · Cerebellum · Purkinje cells · Rat

Introduction

The role of the cerebellum in sensory phenomena is a polemic, even though many converging lines of evidence have pointed to an involvement of the cerebellum in different nociceptive phenomena. For example, somatic noxious stimulation increases cerebellar blood flow in humans (Casey et al. 1994, 1996; Svensson et al. 1997; Iadorola et al. 1998) and rats (Saab et al. 1999). In addition, electrical stimulation of small-diameter afferents in peripheral nerves evokes surface potentials in the cerebellum of cats (Ekerot et al. 1991) and rats (Wu and Chen 1990). On a behavioral level, antinociceptive effects result from electrical stimulation of the brachium conjunctivum in primates (Siegel and Wepsic 1974) and microinjection of morphine in the anterior cerebellum of rats (Dey and Ray 1982). However, the details about the specific neuronal circuitry mediating these effects are elusive. Therefore, after describing an enhancement of spinal cord neuronal responses to visceral noxious stimuli following cerebellar cortical stimulation (Saab et al. 2001), the aim of this study was to show that visceral noxious stimulation by colorectal distension (CRD) modulates the firing rate of cerebellar Purkinje cells. These cells exert an inhibitory control over the deep cerebellar nuclei which constitute the output of the cerebellum.

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Methods

Nine adult Sprague-Dawley rats were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with the guidelines of the National Institutes of Health and the International Association for the Study of Pain. Each rat was initially anesthetized with halothane in a closed Plexiglas chamber (4% for 2 min). A tracheotomy allowed for continuous infusion of halothane (2%) in a mixture of NO_2/O_2 (1:3) during artificial respiration (50–60 strokes/min). The rat was then fixed on a stereotaxic apparatus (Kopf Instruments) and a balloon was gently inserted into the rectum for visceral stimulation by CRD. A craniotomy exposed the caudal vermis and parts of one lateral hemisphere, from which the dura was gently removed. Lukewarm mineral oil (Squibb) was used to cover the exposed cerebellar cortex. Extracellular single-unit recordings were made 0.3–1.0 mm from the surface of the caudal vermis (lobules 7, 8) and lateral hemisphere (crus I, II) using a tungsten microelectrode (0.5-μm-diameter tip, 12 MΩ impedance). The extracellularly recorded action potentials were amplified and fed into a window discriminator, then displayed on an oscilloscope. The outputs from the window discriminator and from the amplifier were led into a data collection system (CED 1401+) and a computer for data compilation as "rate histograms" or "wavemarks" using the Spike 2 software program.

CRD is a well-documented visceral stimulus (Ness and Gebhart 1988; Al-Chaer et al. 1996a, 1996b, 1998). The balloon

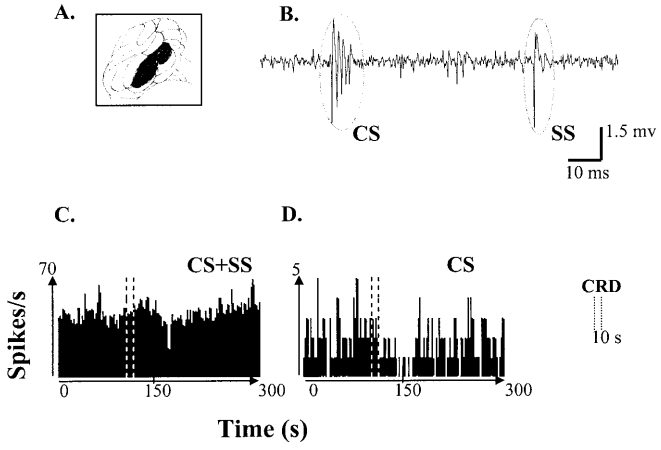

Fig. 1 **A** The recording sites in the cerebellum (*dark shading:* vermis lobules 7, 8, and hemisphere crus I, II). **B** Complex spike (*CS*) and simple spike (*SS*) from the same cell. **C** Overall activity from one Purkinje cell. **D** CS activity of this cell is plotted separately; note the characteristic 1- to 5-Hz frequency. *Vertical and horizontal axes* represent the firing rate in spikes per second and the time in seconds, respectively; *vertical dashed bars* represent the time of CRD application

was constructed from a latex glove finger attached to a 4-cm-long tygon tube that was connected to a manual pump and a pressure transducer to monitor stimulus intensity. Each CRD consisted of a balloon inflation (80 mmHg, considered as noxious) for 10 s. Somatic stimulation consisted of manual squeezing of the ipsilateral hindpaw (using a forceps) or brushing the dorsum of the hindpaw (using a camel hair brush) for 10 s each. Following visceral or somatic stimulation, if the difference between the mean rate of firing 100 s after the onset of a stimulus and that during the 50-s baseline recording that preceded each stimulus was greater than 10%, a response was considered as significant and computed as a percentage change. Subsequently, negative and positive responses were grouped separately and compared with a standardized baseline measure (100%) using a paired *t*-test.

Purkinje cells were identified based on the occurrence of complex spikes (Fig. 1B), the relative size and frequency of complex versus simple spikes (Fig. 1C, D), and the depth of recording (0.3–0.6 mm from the surface and near the Purkinje cell layer; for a thorough review, refer to Eccles et al. 1967). At the end of the experiment, small lesions were made by passing a direct current (15–20 μA for 5 s) through the microelectrode to mark the recording site. The tissue was then preserved in 10% neutral formalin and prepared for sectioning (50 μm thick) using a sliding microtome, and the neurons were stained with neutral red dye.

Results

A total of 82 cells were isolated from the caudal vermis and adjacent lateral hemisphere (Fig. 1A). Spontaneous, complex spike activity characteristic of Purkinje cells was detected in 75 cells (Fig. 1B, D). The other 7 neurons could not be identified on the basis of complex spike activity. After the identification of a Purkinje cell, the spontaneous background activity was monitored for at least 100 s prior to testing the effect of peripheral stimulation.

Visceral stimulation

Although an interesting oscillatory behavior was observed in some Purkinje cells (Figs. 2D, 2, 3A, 5), only cells with a relatively stable baseline activity are summarized in Fig. 2E (for description of a similar oscillatory behavior, refer to Hartmann and Bower 1998). Of the 75 neurons identified as Purkinje cells, 19 exhibited either a significant decrease (n=13, Fig. 2A, 2, C, 2) or a significant increase (n=6, Fig. 2A, 1, C, 1) in firing rate following CRD, whereas 5 showed biphasic responses (an increase followed by a decrease in firing rate; Fig. 2D). Typical responses of the spinal cord lamina X neurons occur essentially within the period of visceral stimulation (Al-Chaer et al. 1996a, 1996b, 1998; Garrison and Foreman 1997). However, certain responses observed in this study occurred following the stimulus and persisted long after its termination. These responses were therefore classified as "delayed" (n=9). They occurred with a mean delay time of 17±7 s following CRD (Fig. 2C1, 2), whereas other responses were classified as "early" (n=10) when the delay was less than 1 s (Fig. 2A). The stimulus was often applied twice when the delay was prolonged (Figs. 2C, 2, 2′and D, 2, 2′), to ensure that the change in activity was linked to the stimulus.

Somatic stimulation

Brushing of the dorsum of the hindpaw often elicited weak but significant responses in the same Purkinje cells described above (n=6), whereas squeezing (n=6) or capsaicin injection (1%, 0.03 ml i.d., n=4) in the plantar aspect of the hindpaw resulted in more prominent responses (for examples, refer to Fig. 3A). In seven isolated cells where a complex spike could not be detected ("unidentified" cells), squeezing of the somatic receptive field in the face or forelimb regions elicited clear excitatory responses (other somatic or visceral stimuli were not effective). These responses were strictly correlated in time with the stimulus (Fig. 3B).

Discussion

The results suggest that the cerebellar Purkinje cells respond to visceral nociceptive and other sensory information in the form of early and delayed changes in their firing rate. These findings are in agreement with recent data describing neurons in the lateral reticular nucleus in the medulla that receive visceral nociceptive input and project to the cerebellar cortex (Ness et al. 1998). However, the pathways involved and the role played by the cerebellum in nociceptive processing are still unclear.

Studying the effect of noxious stimulation on the activity of Purkinje cells contributes to a better understanding of cerebellar involvement in nociceptive phenomena. Purkinje cells exert a major inhibitory control over neu-

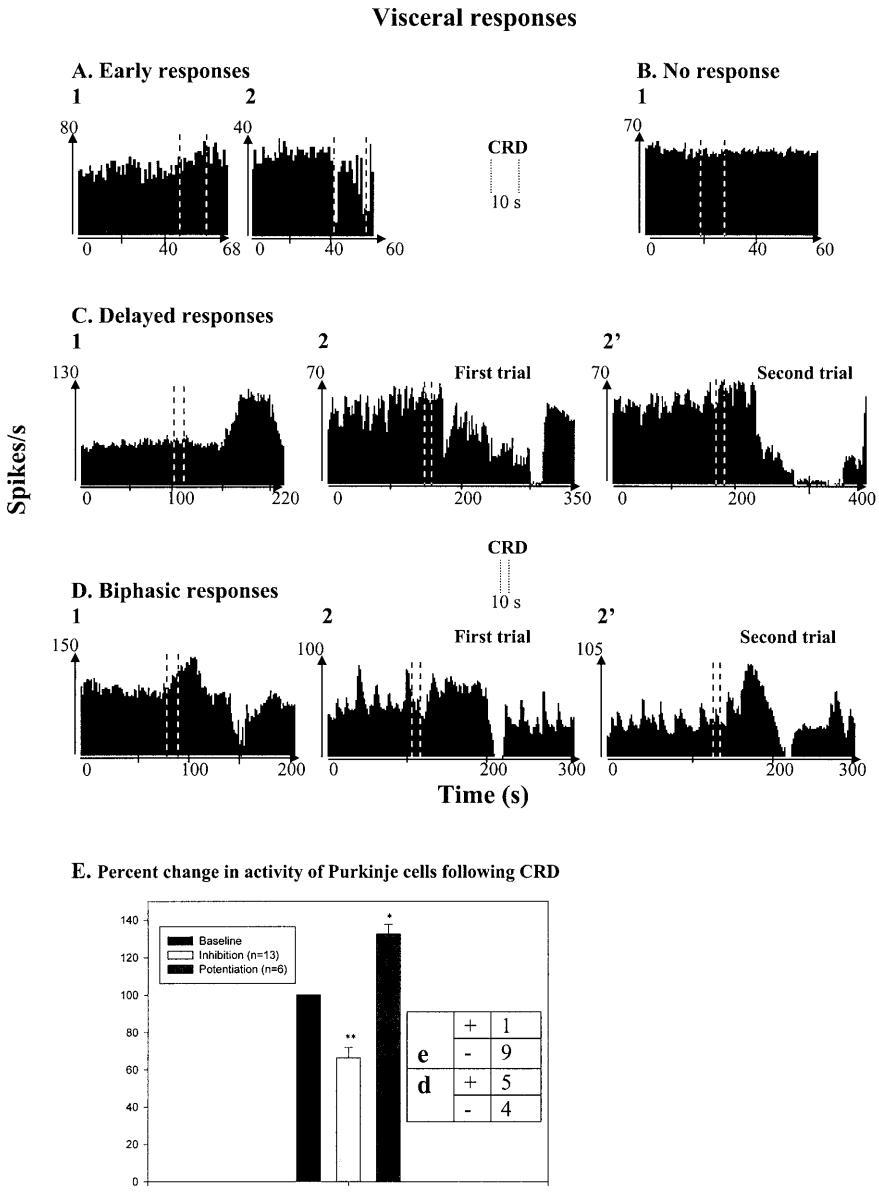

Fig. 2 A–D Peristimulus-time histograms from Purkinje cells showing responses to colorectal distension (*CRD*). **A** Early responses to CRD (*1*, increase; *2*, decrease). **B** No response to CRD. **C** Delayed responses (*1*, delayed increase; *2*, *2′*, consecutive trials showing delayed decreases in activity recorded from the same cell). **D** Biphasic responses. Biphasic response in one cell (*1*); consecutive trials showing biphasic responses recorded from the same cell (*2*, *2′*; note the oscillatory behavior). *Vertical and horizontal axes* represent the firing rate in spikes/s and the time in seconds, respectively; *vertical dashed bars* represent the time of CRD application. **E** Percentage change in rate (spikes per second) following CRD. The change was calculated as the difference between the mean rate of activity 100 s following, and 50 s before, CRD. Only changes of more than 10% are included (n_{total}=19). *Inset table* classifies the cells into different categories (*e* early, *d* delayed, + increase, – decrease). Baseline for included cells was standardized to 100% and significance was calculated using a paired *t*-test (**P*<0.01; ***P*<0.0005)

rons in the underlying cerebellar nuclei. These nuclei in turn represent the output of the cerebellum and mediate its functions, including the regulation of motor, sensory, and cognitive activities (Holmes 1922, 1939; Bloedel and Bracha 1995; Fiez 1996; Houk 1997; Schmahmann and Pandya 1997). An interesting speculation is that nociceptive input may modulate one or more of these functions through Purkinje cells. In fact, if cerebellar cortical stimulation enhances spinal neuronal responses to visceral noxious stimuli (Saab et al. 2001), the results of this study show that, when Purkinje cells responded to similar stimuli, inhibition occurred in the majority of cases (13/19 cells). Therefore, it is tempting to ascribe an antinociceptive role to the deep cerebellar nuclei, which are anatomically connected with the brain stem analgesic centers (Palay and Chan-Palay 1982; Mendlin et al. 1996; Voogd and Glickstein 1998), as a part of a negative feedback circuit.

The temporal pattern of the responses reported in this paper is different from that characteristic of, for example, postsynaptic dorsal column, thalamic, and spinothalamic cells (for a review, refer to Willis and Coggeshall 1991). Delayed neuronal responses have been reported before (Oniani et al. 1972; McDevitt et al. 1987; Dean and Boulant 1992; Hesslow 1994), and the delay was attributed to indirect projections, multiple synapses, and indirect effects such as those of hormonal or cardiovascular origin. Furthermore, this delay does not seem to depend on the anesthetic agent; extracellular recording from Purkinje cells in Crus I and II from anesthetized rats using Nembutal (0.55 mg/kg i.p.) instead of halothane revealed comparable responses to brushing, pinching, or applying a pinprick to the ipsilateral hindlimb (unpublished observation).

The results of the noxious somatic stimuli used in this study (squeezing of the skin of the hindpaw or other ar-

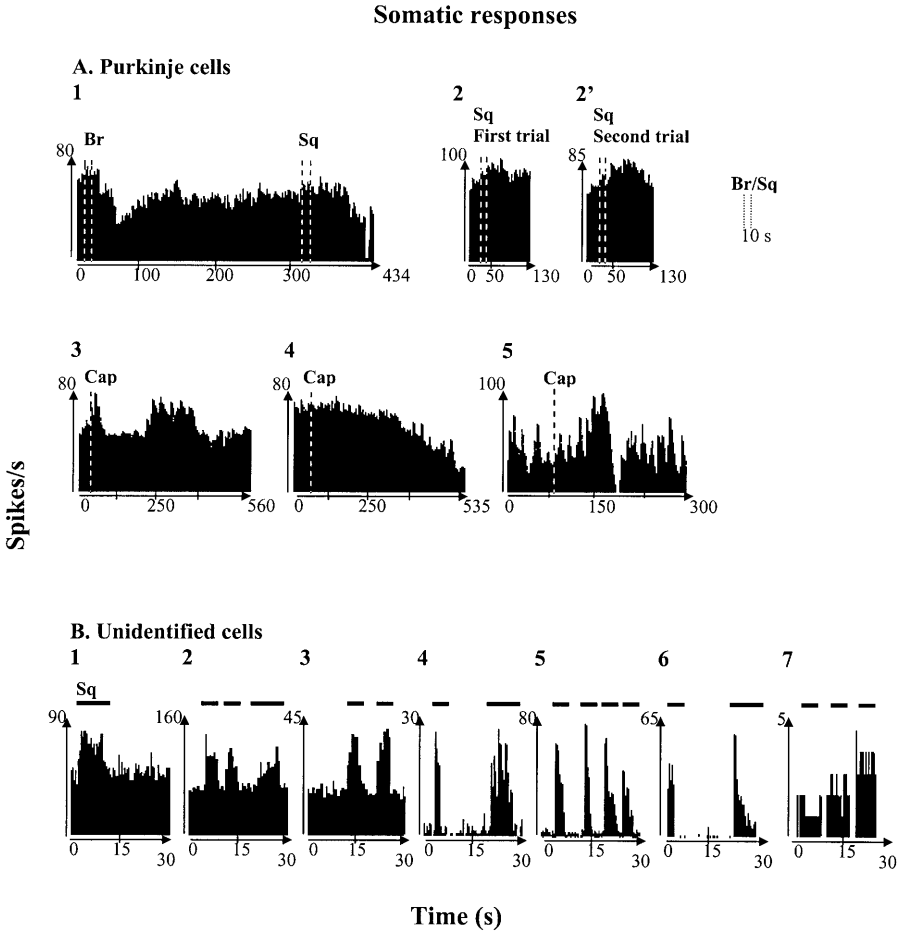

Fig. 3A, B Peristimulus-time histograms from Pukinje (**A**) or unidentified cells (**B**); responses to somatic stimulation. **A** *Br*, *Sq*, and *Cap* denote brushing, squeezing, and capsaicin injection in the ipsilateral hindpaw, respectively (*2*, *2′*, consecutive trials showing responses to Sq recorded from the same cell; the Purkinje cell in *5* is the same shown in Fig. 2D, 2, 2′). **B** *Horizontal bars* represent the time (*shorter bar*, 5 s; *longer bar*, 10 s) when an ipsilateral receptive field (*1*, *3*; *2*, *4*, *6*; *5*, and *7* are, respectively, the forepaw, upper lip, lower lip, and lower tooth) was squeezed using a forceps. In *7*, the three peaks represent the responses to tapping using a forceps on an ipsilateral lower tooth. The response had increasing frequencies of 1, 2, and 3 spikes/s, respectively. *Vertical and horizontal axes* represent the firing rate in spikes per second and the time in seconds, respectively; *vertical dashed bars* in **A** represent the time of somatic stimulation (Br, Sq, or Cap injection)

eas) support the idea that the change in neuronal activity may not be specific to one sensory modality (such as visceral nociception), but rather represents a response to a general state of nociception. However, this assumption is weakened based on the responses elicited by gentle brushing of the skin, which further indicates that an individual Purkinje cell may process information that is probably related to a general sensory state that is unlikely due to hormonal or cardiovascular epiphenomena (for characterization of pseudoaffective reflexes in the rat following CRD, refer to Ness and Gebhart 1988). Accordingly, it is possible that the involvement of the cerebellum in many functions may be manifested in the information-encoding capability of individual Purkinje cells. In addition, since the Purkinje cells studied were isolated from different sagittal planes of the posterior cerebellum, the visceronoxious input may not be viscerotopically organized (for a review of cerebellar somatotopy, refer to Leergaard et al. 2000).

In a behavioral context, pain involves learning and memory processes stored in the nervous system (Fuchs and Melzack 1997; Al-Chaer et al. 2000; Sandkuhler 2000). On the other hand, the cerebellum retains automata of different motor tasks for prolonged periods of time (Bloedel and Bracha 1995; Devor 2000). Therefore, the delay in some of the responses observed in this study may well represent a form of sensory information retention (one capsaicin injection elicited a neuronal response that lasted for more than 30 min, concurring with the human psychophysical experience; LaMotte et al. 1991).

In conclusion, cerebellar Purkinje cells respond to visceral noxious stimuli in different temporal patterns that often require repeated measurements to ensure a stimulus-response correlation. The single trials are more readily interpreted when classified and averaged (Fig. 2E). It should be assumed that, following a single stimulus, all of the responses described above are likely to occur simultaneously in many Purkinje cells, thus perhaps encoding many aspects of a single nociceptive event by recruiting similar or different pathways yet to be studied. Multielectrode recording and advanced computational techniques are deemed necessary to further our knowledge in this regard.

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