

STIMULATION OF CAT CUTANEOUS NOCICEPTIVE C FIBRES CAUSING TONIC AND SYNCHRONOUS ACTIVITY IN CLIMBING FIBRES

BY C.-F. EKEROT, O. OSCARSSON AND J. SCHOUENBORG

*From the Institute of Physiology and Biophysics, Lund University, Sölvegatan, 19,
S-223 62 Lund, Sweden*

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SUMMARY

1. The input from cutaneous nociceptors to climbing fibres projecting to the forelimb area of the C3 zone in the cerebellar anterior lobe was examined in barbiturate-anaesthetized cats. Climbing fibre responses were simultaneously recorded in single Purkinje cells and as field potentials from the cerebellar surface close to these cells.

2. The cutaneous receptive field of the climbing fibres studied were located on the ipsilateral forelimb. All climbing fibres were activated by both non-noxious tactile stimulation and noxious pinch of the skin. The location of the receptive field and the distribution of sensitivity in the receptive field appeared to be identical for noxious and tactile stimuli.

3. A phasic response in the climbing fibres was evoked by either short- or long-lasting non-noxious pressure applied to their cutaneous receptive field. By contrast, all climbing fibres studied were strongly and tonically activated (up to 4–11 Hz for the duration of the stimulation) by sustained noxious pinch in the most sensitive area of their receptive fields.

4. Experiments with anodal block of impulse conduction in myelinated fibres indicated that a major input to climbing fibres during sustained noxious pinch originates from nociceptive C fibres.

5. Sustained noxious pinch of the skin evoked large field potentials on the cerebellar surface. These field potentials were evoked simultaneously with climbing fibre responses in single Purkinje cells and were due to synchronous activation of many climbing fibres. These field potentials and discharges in single climbing fibres were elicited from the same area of the skin suggesting that many of the synchronously discharging climbing fibres have the same receptive field on the skin.

INTRODUCTION

In a previous investigation it was demonstrated that the majority of climbing fibres projecting to the C3 zone in the anterior lobe of the cerebellum are activated by electrical stimulation of cutaneous C fibres (Ekerot, Gustavsson, Oscarsson & Schouenborg, 1987). In the present study it was found that noxious mechanical

stimulation of the skin causes a powerful activation of climbing fibres projecting to the C3 zone. The generation of this response by impulses in nociceptive C fibres was investigated. It was investigated whether impulses in nociceptive C fibres could generate this response. In addition, the characteristics of the cutaneous receptive fields of climbing fibres were examined using noxious mechanical stimuli and tactile stimuli.

METHODS

The experiments were performed on fourteen cats under pentobarbitone anaesthesia. Some of these animals were also used in a study of climbing fibre responses evoked by electrical stimulation of C fibres which should be consulted for the methods employed (Ekerot *et al.* 1987). Only additional procedures will be described here.

Manual mechanical stimulation of the skin included light tapping with the tip of a pair of forceps, maintained firm non-noxious pressure on the skin and noxious pinch. In the latter two cases a small flap of skin (area approximately 3 mm²) was placed within the grip of a pair of flat forceps. The noxious stimulation was preceded by maintained non-noxious pressure held for 3–5 s. The non-noxious pressure employed was not painful, whereas the noxious pinch evoked moderate pain when tested on our skin.

In four experiments the skin was stimulated during selective anodal block of impulse conduction in A fibres in the superficial radial nerve (Ekerot *et al.* 1987). In such experiments it was necessary to cut all the other nerves (except the superficial radial nerve) supplying the distal half of the forelimb to ensure the efficacy of the block.

RESULTS

Activity in climbing fibres projecting to the forelimb area of the C3 zone was recorded simultaneously in Purkinje cells ($n = 31$) and as surface field potentials in the sagittal plane of the Purkinje cell. Most surface recordings were made within 0.5 mm of the Purkinje cell.

Partially denervated preparation

In the typical example shown in Fig. 1 *A* noxious pinch of the dorsal skin of the second digit resulted in a tonic, high-frequency, climbing fibre activity in the Purkinje cell and in the generation of large field potentials at the cerebellar surface. The responses lasted for the duration of the stimulation (7.5 s). The climbing fibre responses in the Purkinje cells often occurred simultaneously with the large field potentials recorded at the surface. Hence the field potentials were evoked by synchronous discharges in many climbing fibres. The amplitude of the field potentials varied during the noxious stimulation indicating that the number of simultaneously activated climbing fibres differed from time to time.

Anodal block of impulse conduction in myelinated fibres in the ipsilateral superficial radial nerve was used to determine if impulses in nociceptive C fibres contributed to the climbing fibre activity evoked by noxious stimulation of the skin. The recordings of climbing fibre activity in Fig. 1 *A* were continued in *B* during anodal block of myelinated fibres. It can be seen that the features of the climbing fibre activity were unchanged during the block. The mean frequency of the climbing fibre discharge during block in the five Purkinje cells tested was similar to the control values (3.5 and 2.8 Hz, respectively).

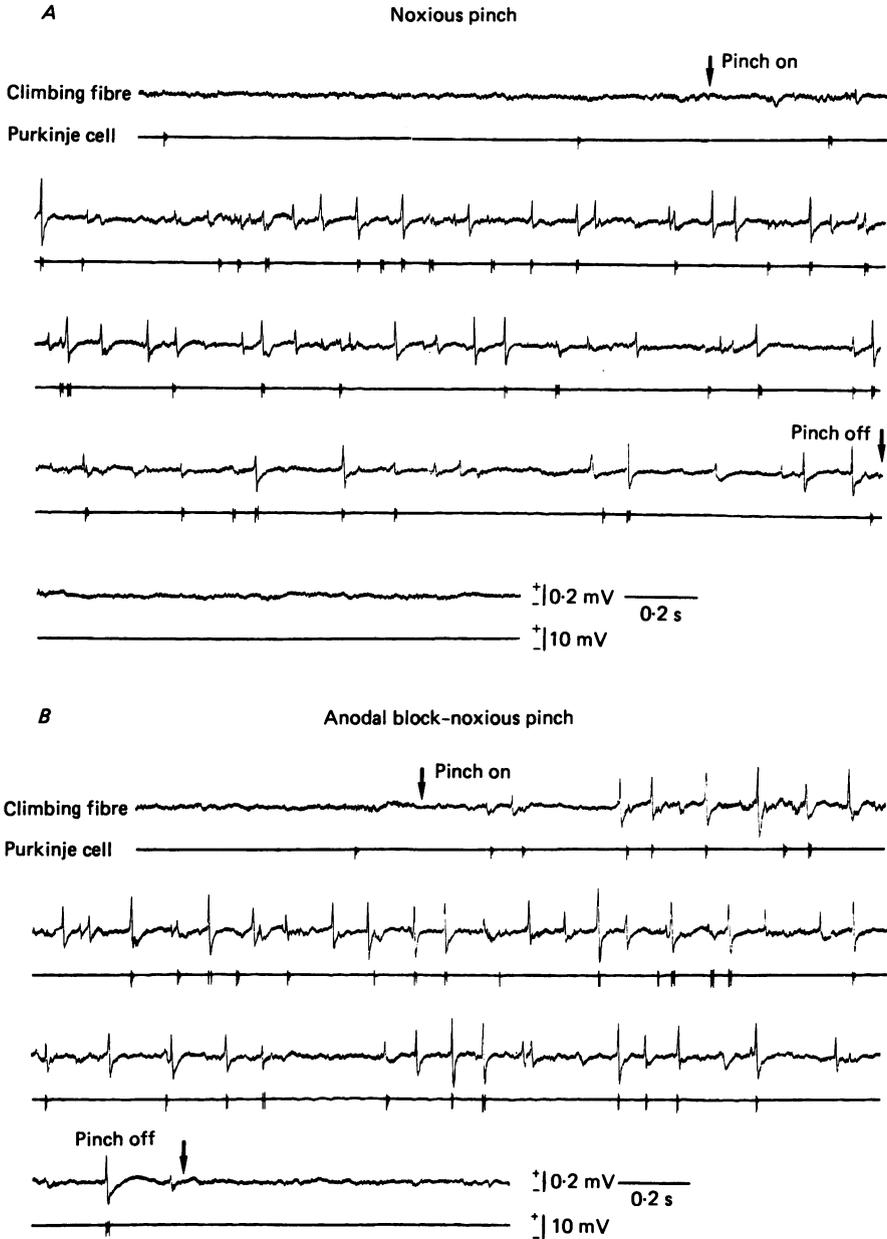


Fig. 1. Simultaneous recording of climbing fibre responses in a Purkinje cell and climbing-fibre-evoked field potentials on the cerebellar surface at a site close to and in the same sagittal plane as the Purkinje cell. *A*, noxious pinch was applied to the dorsal part of the skin of the second digit of the ipsilateral forelimb as indicated. The distal part of the forelimb was partially denervated by cutting all nerves except the superficial radial nerve. *B*, climbing fibre activity evoked by noxious pinch, recorded during anodal block of impulse conduction in A fibres in the superficial radial nerve.

Preparation with intact innervation

The receptive fields of single climbing fibres ($n = 26$) were studied in experiments with intact innervation of the forelimbs. The cutaneous receptive field for noxious and tactile stimuli (light tapping and non-noxious pressure) had similar locations on the ipsilateral forelimb. The receptive field often consisted of two to three digits, and

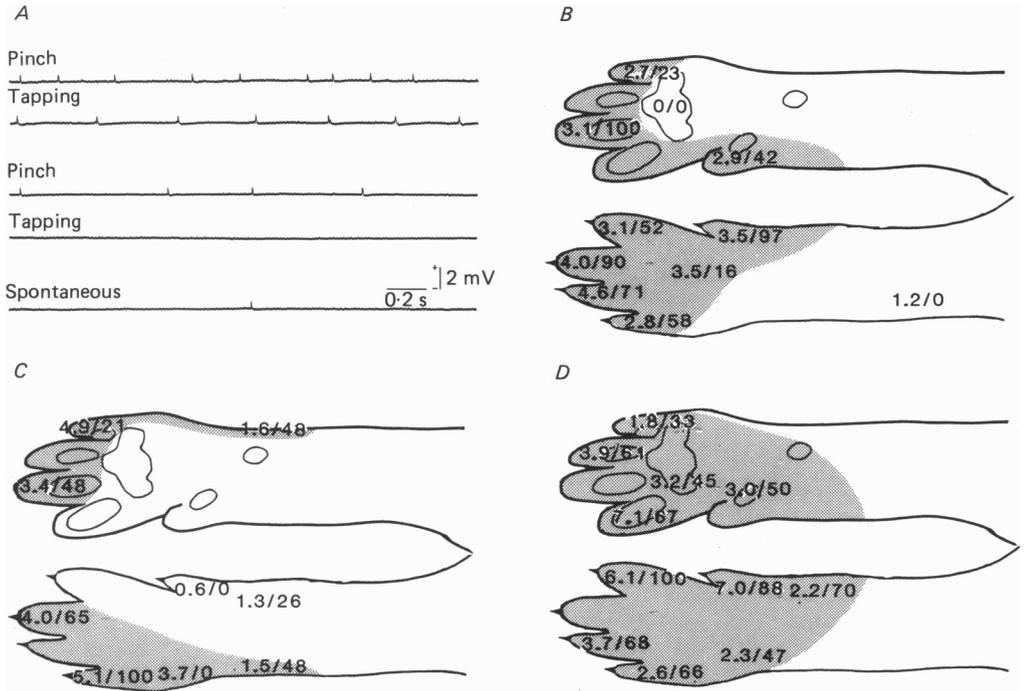


Fig. 2. Receptive fields of climbing fibres as determined using manual noxious pinch and light tapping. *A*, samples of climbing fibre responses in one Purkinje cell on noxious pinch and repetitive tapping within the most sensitive area (top records) and from a point outside the receptive field (bottom records). The extent of the receptive field is indicated by the shaded area in *B*. The mean frequency of climbing fibre responses on sustained noxious pinch and the number of climbing fibre responses during repetitive light tapping given as a percentage of the number of stimulations is indicated for eleven spots. *C* and *D*, two further typical examples of receptive fields of climbing fibres projecting to the C3 zone.

part of the dorsal and/or ventral surface of the paw and forelimb. The mean frequency of climbing fibre discharges in Purkinje cells evoked by noxious pinch in the most sensitive area was 5.7 Hz (range: 3.3–11.1 Hz; spontaneous activity was usually 0.5–1.5 Hz). Following the termination of the noxious pinch a pause in the spontaneous climbing fibre activity for several seconds was noted in most Purkinje cells. By contrast sustained non-noxious pressure applied to the most sensitive area usually evoked only a single climbing fibre discharge at the start and at the end of the stimulation. The climbing fibre response to light tapping in the most sensitive area usually consisted of one response per tap.

In fifteen Purkinje cells the receptive fields of the climbing fibres were assessed by counting the responses to tactile and noxious stimulation. The receptive field of the two modalities appeared to be identical. Fig. 2 shows three examples of receptive fields. The responses evoked from the most sensitive area and from a point outside

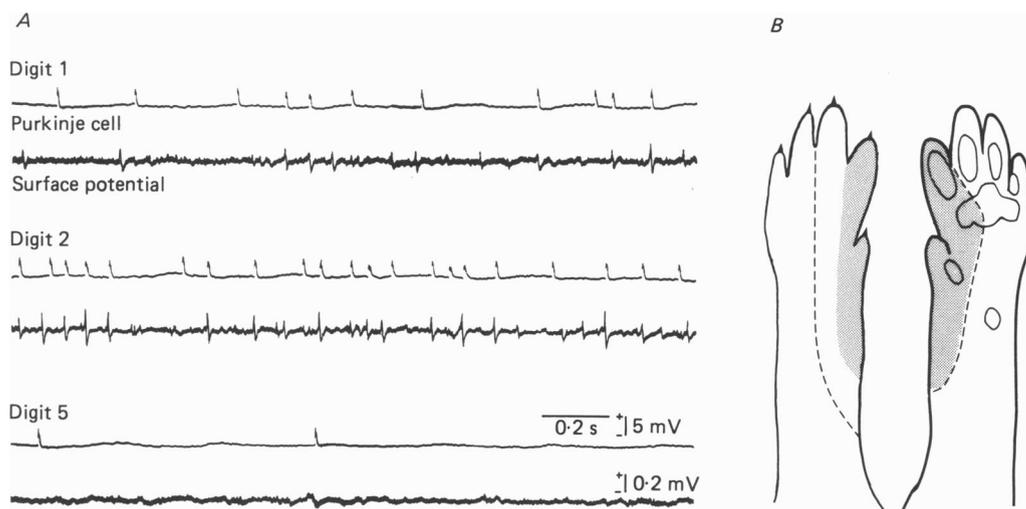


Fig. 3. Climbing fibre activity recorded from Purkinje cell and from the surface. *A*, climbing fibre responses evoked on noxious pinch of digits 1, 2 and 5. *B*, receptive field of the climbing fibre responses shown in *A*. Shaded area indicates the receptive field of the climbing fibre and the area within the interrupted line indicates the receptive field of the surface-evoked field potentials.

the receptive field by sustained noxious pinch and by repetitive tapping in one unit are given in Fig. 2*A*. Since there was always some fluctuation in excitability of the climbing fibres studied the apparent extent of the receptive field varied. Thus a long recording period (about 1 h) was required to determine the extent of the receptive field. The mean frequency of the climbing fibre responses during sustained (5–10 s) noxious pinch and the number of climbing fibre responses evoked by repetitive tapping are indicated in Fig. 2*B–D*. There was usually one or several foci with particular high sensitivity within the receptive field (Fig. 2). As can be seen in Fig. 2*B* and *C* the gradient of sensitivity within the receptive field was very steep in some areas.

Fig. 3*A* shows the climbing fibre responses in one Purkinje cell and the climbing fibre field potentials evoked in the same sagittal plane by noxious pinch of digits 1, 2 and 5. For both field potentials and unit activity the maximal responses were much larger from digit 2 than from digit 1 and no climbing fibre response could be evoked from digit 5. Fig. 3*B* shows the receptive field of the climbing fibre responses recorded from the Purkinje cell (shaded area) and from the surface (interrupted line). Climbing-fibre-evoked responses in single Purkinje cells and climbing-fibre-evoked field potentials recorded in the same sagittal plane were always elicited from the same area of the skin.

DISCUSSION

The present investigation demonstrates that climbing fibres projecting to the C3 zone are strongly activated by noxious stimulation of the skin. The frequency of climbing fibre responses elicited by noxious stimulation was often higher than 4 Hz, which has been assumed to be the maximal frequency in behaving animals (see Ito, 1984).

The finding that an intense and prolonged discharge in climbing fibres was evoked by noxious pinch also during a selective block of impulse conduction in A fibres shows that impulses in C fibres are sufficient to generate this climbing fibre response. There was no tonic climbing fibre response to sustained non-noxious pressure. Out of the three main classes of cutaneous C fibres which have been identified in the cat, nociceptors, low-threshold mechanoreceptors and thermoreceptors (Iggo, 1959, 1960; Bessou & Perl, 1969; Bessou, Burgess, Perl & Taylor, 1971; Beck, Handwerker & Zimmermann, 1974; see also Willis & Coggeshall, 1978), only nociceptors would be expected to be preferentially activated by a noxious mechanical stimulus. Furthermore, the low-threshold unmyelinated mechanoreceptors usually show a pronounced adaptation to sustained pressure (Iggo, 1960; Bessou *et al.* 1971). It is not known if nociceptive A δ fibres give a contribution to the tonic climbing fibre response during noxious stimulation.

The large field potentials evoked by noxious stimulation often occurred simultaneously with the climbing fibre discharge in single Purkinje cells. Hence, these field potentials reflect synchronous activation of large numbers of climbing fibres. A possible mechanism contributing to the synchronization is the electronic coupling of neurones in the inferior olive by gap junctions (Llinás, Baker & Sotelo, 1974; Sotelo, Llinás & Baker, 1974; Llinás & Yarom, 1980), which may convert the tonic asynchronous activity in C fibres into synchronous discharges of olivary neurones.

The tonic activity in climbing fibres evoked by sustained noxious stimulation differs from previous reports on the climbing fibre activity evoked by adequate stimulation of other peripheral receptors (Eccles, Sabah, Schmidt & Taborikova, 1972; Leicht, Rowe & Schmidt, 1973; Rushmer, Roberts & Augter, 1976; Gellman, Gibson & Houk, 1985). In most cases a phasic discharge in the climbing fibres has been observed at the start and the end of the stimulation. These observations have led to the hypothesis that climbing fibres serve as 'event detectors' (Rushmer *et al.* 1976). This seems unlikely since the frequency of the tonic climbing fibre discharge evoked by noxious stimulation was a function of stimulation intensity. Furthermore, the convergence of tactile A β fibres, nociceptive C fibres, and in most cases, also A δ fibres (Ekerot *et al.* 1987), on climbing fibres projecting to the C3 zone indicates that these climbing fibres do not carry information about the kind of receptors activated (see also Oscarsson, 1973), nor about the precise timing of the peripheral events. Rather, the restricted extent of the multimodal receptive fields seems to indicate that the climbing fibres carry spatial information from the periphery.

It has been suggested that spino-olivary pathways carry information about activity in segmental reflex paths (Oscarsson, 1973). Since the receptive fields of the climbing fibre responses recorded from the surface were very similar to those of individual climbing fibres it is likely that the receptive fields of many of the climbing

fibres projecting to a sagittal plane in the C3 zone is nearly identical. If these assumptions are correct it is conceivable that these climbing fibres receive information from a very limited number of segmental reflex paths.

The finding of a powerful nociceptive input to climbing fibres is interesting with regard to the proposed role of the cerebellum and climbing fibres in motor learning (for references see Ito, 1984). The somatotopically organized powerful nociceptive C-fibre input to climbing fibres is consistent with the hypothesis that these fibres carry information about errors in motor performance (Miller & Oscarsson, 1970; Ito, 1972; Andersson & Nyquist, 1983; Andersson & Armstrong, 1985; Gellman *et al.* 1985). In most situations a movement resulting in activation of nociceptors would be erroneous. Such motor errors may be particularly frequent following an inflammatory tissue reaction. It has been shown that climbing fibre impulses induce long-lasting plastic changes in simultaneously activated synapses between parallel fibres and Purkinje cells (Ito, Sakurai & Tongroach, 1982; Ekerot & Kano, 1985). It is likely that the strong tonic activity evoked in climbing fibres by noxious stimulation of the skin would be particularly effective in inducing such long-lasting changes.

The strong tonic climbing fibre activity evoked by noxious stimulation of the skin would also be expected to cause immediate drastic changes in the motor control exerted by the cerebellum. It is likely that these climbing fibre responses result in a strong synchronous inhibition of cerebellar nuclear cells, resembling that occurring in harmaline-treated animals (Linás & Volkind, 1973). Also, stimulation of climbing fibres at a frequency of 8–10 Hz has been shown to result in a pronounced depression of single spike activity in Purkinje cells lasting several minutes after the end of the stimulation (Rawson & Tilokskulchai, 1981) and recent investigations show that this effect can be obtained at stimulation frequencies as low as 3–4 Hz (G. Andersson & G. Hesslow, personal communication). Hence, at the end of the noxious stimulation a period of reduced inhibition of cerebellar nuclear cells would follow. These immediate effects may be related to the inhibition of on-going movements, which usually follows a noxious stimulus (Sherrington, 1910).

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