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*Published in:*  
Journal of Physiology

*DOI:*  
[10.1113/jphysiol.2001.013507](https://doi.org/10.1113/jphysiol.2001.013507)

2002

[Link to publication](#)

*Citation for published version (APA):*

Garwicz, M., Levinsson, A., & Schouenborg, J. (2002). Common principles of sensory encoding in spinal reflex modules and cerebellar climbing fibres. *Journal of Physiology*, 540(Pt 3), 1061-1069.  
<https://doi.org/10.1113/jphysiol.2001.013507>

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# Common principles of sensory encoding in spinal reflex modules and cerebellar climbing fibres

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An important step towards understanding the function of olivo-cerebellar climbing fibres must be to clarify what they signal. We suggest that climbing fibres projecting to paravermal cerebellum mediate highly integrated sensorimotor information derived from activity in spinal withdrawal reflex modules acting on single forelimb muscles. To test this hypothesis, cutaneous nociceptive receptive fields of spinal reflex modules were mapped and compared to those of climbing fibres. Quantitative methods were used both for mapping and for comparing receptive fields. The organization of muscle afferent input converging on individual climbing fibres was analysed in the light of results from receptive field comparisons. Individual cutaneous receptive fields in the two systems were readily matched. Matched pairs were highly similar with regard to detailed distributions of sensitivity: correlation coefficient  $r = 0.85$ ; overlap of receptive field foci 72 % (average values). The olivary targets of muscle afferents from a given muscle were mainly climbing fibres with cutaneous receptive fields similar to that of the muscle itself, but to a lesser extent also other climbing fibres. In conclusion, paravermal climbing fibres apparently convey information integrating (i) cutaneous input to an individual spinal withdrawal reflex module, (ii) muscle afferent input from the output muscle of that module and (iii) muscle afferent input from muscles that constitute the output of functionally related modules. This suggests that an individual climbing fibre signals cutaneous sensory events reflecting activity of a single muscle conditional upon the functional state of the muscle itself and that of functionally related muscles.

(Resubmitted 6 November 2001; accepted after revision 12 February 2002)

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Despite the fact that the olivo-cerebellar climbing fibre system is pivotal to cerebellar function there is to date little consensus about what climbing fibres actually do (Simpson *et al.* 1996). An important step towards a better understanding of climbing fibre function must be to clarify the nature of afferent signals conveyed by inferior olivary neurons. Analyses of inferior olivary activity in relation to various parameters of motor behaviour in different motor tasks have provided a multifaceted account of when, why and how olivary neurons are activated (Gilbert & Thach, 1977; Gellman *et al.* 1985; Andersson & Armstrong, 1987; Ojakangas & Ebner, 1994; Welsh *et al.* 1995; Kitazawa *et al.* 1998). However, in the context of limb movement control the precise conditions for olivary activation (and hence the significance of the information that the climbing fibres provide to the cerebellum) remain unclear.

Complementary to the approach of relating inferior olivary activity to behavioural parameters is the analysis of how sensory input is encoded in the olivo-cerebellar system and how it is distributed and processed in the cerebellar cortex. The aim is to derive from this analysis what type of information the climbing fibres mediate and how this

information reflects their function. The analysis of sensory encoding presented here concerns a well-defined subset of climbing fibres and builds upon studies of spatial organization of cutaneous receptive fields (Ekerot *et al.* 1991a; Jörntell *et al.* 1996; Garwicz *et al.* 1998), patterns of convergence between muscle and cutaneous afferents (Jörntell *et al.* 1996) and olivo-cortico-nuclear connectivity (Ekerot *et al.* 1991a; Garwicz & Ekerot, 1994; Garwicz *et al.* 1996; Apps & Garwicz, 2000). These climbing fibres originate in the rostral part of the dorsal accessory olive and innervate the C1, C3 and Y zones in the paravermal cerebellum (Voogd & Bigaré, 1980; Voogd, 1982; Andersson *et al.* 1987). Via projections to the deep cerebellar nucleus interpositus anterior (e.g. Trott & Armstrong, 1987) these zones influence the rubrospinal and, indirectly, the corticospinal tracts. Several findings indicate that this subset of climbing fibres conveys highly integrated sensorimotor information, possibly derived from activity in simple spinal reflex networks. This would certainly be compatible with the general notion put forward by Oscarsson (1973), that pathways to the inferior olive monitor activity in spinal motor centres.

First, individual olivary neurons in the forelimb area of this system receive convergent input from several different types of cutaneous afferent fibres (Ekerot *et al.* 1987a) and from group II muscle afferents (Jörntell *et al.* 1996). Second, the spatial organization of cutaneous receptive fields has a number of characteristics that are at odds with typical features of 'classical' sensory systems. The receptive field borders commonly coincide with joints and the receptive field focus is often eccentrically located within the receptive field (Ekerot *et al.* 1991a,b). As a population, the receptive fields can be divided into a number of classes and subclasses defined by distinct spatial characteristics. Hence, the representation of the forelimb skin is in some sense discontinuous, with discrete rather than gradual changes in receptive field characteristics. Despite the overlap with respect to skin areas covered by different receptive field subclasses the representation does not cover the surface of the limb evenly. In addition, climbing fibres belonging to the same receptive field subclass terminate along sagittally oriented cortical strips, termed microzones. Each microzone is exclusive with respect to type of climbing fibre receptive field and neighbouring microzones often have rather dissimilar receptive fields (Ekerot *et al.* 1991a,b). The clustered nature of the receptive field population and the microzonal organization of subclasses are preserved in the cortical projection to nucleus interpositus anterior, where microzones with similar receptive fields converge but those with dissimilar ones terminate on separate groups of neurons (Garwicz & Ekerot, 1994; Apps & Garwicz, 2000).

All these are features of a modular system, i.e. a system subdivided into distinct units with at least some functional independence. What does the modularity reflect and what is its function? Since the number of modules is in close accord with the number of muscles in the forelimb (Reighard & Jennings, 1966; Garwicz & Ekerot, 1994), one may speculate that individual olivary neurons convey information reflecting some aspect of the action of specific single muscles. For instance, the encoding and processing of information may reflect the relative efficiency with which muscular output affects the position of the skin surface.

To test whether the information conveyed by individual olivary neurons is encoded in a way that is compatible with principles of sensorimotor transformation in simple spinal reflex networks, we compared cutaneous receptive fields of climbing fibres to those of spinal withdrawal reflexes (Schouenborg & Kalliomäki, 1990). The withdrawal reflex system has a modular organization such that sensory input to a given muscle is provided specifically from the area of skin that is actually displaced upon contraction of the muscle itself, when the limb is in the standing position (Schouenborg & Weng, 1994). In a given reflex module, the sensitivity within the receptive field is

finely graded, reflecting the spatial distribution of withdrawal efficacy of the muscle. Hence, the individual muscle in the adult animal (see also Holmberg & Schouenborg, 1996) has a highly characteristic cutaneous receptive field that amounts to a sensory encoding of the action of that muscle (Schouenborg & Weng, 1994). While withdrawal reflexes have been thoroughly investigated in the rat hindlimb (Schouenborg & Kalliomäki, 1990) and recently also in the cat hindlimb (Levinsson *et al.* 1999), data on the cat forelimb are insufficient for a meaningful comparison with the extensive pool of forelimb-related climbing fibre receptive fields. Therefore, in order to allow an evaluation of functional relatedness between climbing fibres and withdrawal reflexes, we report here an extension of the pilot investigation of withdrawal reflex modules in the cat forelimb (Levinsson *et al.* 1999) and carry out novel analyses of data derived from databases obtained in two previous studies (Ekerot *et al.* 1991a; Jörntell *et al.* 1996).

Does the receptive field of a given climbing fibre subclass correspond to the cutaneous receptive field of a withdrawal reflex module and therefore, by analogy, to the skin area affected by the action of a particular single muscle? If so, to what extent does this particular muscle provide afferent input to that same subclass of climbing fibres? The portions of the climbing fibre system and the withdrawal reflex system that have so far been investigated have well-documented modular organizations and highly specific spatial distributions of sensitivity within their receptive fields. Hence, a high degree of similarity between climbing fibre and forelimb withdrawal reflex receptive fields would strongly indicate that encoding of sensory input is indeed equivalent in the two systems.

## METHODS

### Animals anaesthesia and preparation

The experimental procedures were reviewed and approved by the Malmö/Lund Ethics Committee for Animal Experimentation (application no M164-97) and the District Court of Lund. The experiments were carried out on eight purpose-bred adult cats of both sexes (2.8–4.7 kg) under general anaesthesia which was additionally supplemented by infiltration of a local anaesthetic (see below). No muscle relaxants were used. Where indicated, data derived from databases obtained in two previous studies (Ekerot *et al.* 1991a, Jörntell *et al.* 1996) were also used.

The animals were anaesthetized with pentobarbital (40 mg kg<sup>-1</sup> i.p. with supplementary doses i.v. as required). The mean arterial blood pressure, end expiratory CO<sub>2</sub> concentration and rectal temperature were monitored throughout the experiment (90–130 mmHg, 4.0–5.5 % and 37.5–38 °C, respectively). The level of anaesthesia was characterized by a stable blood pressure, constricted pupils, slow regular breathing and general muscle atonia, except in respiratory muscles. Concentrations of pentobarbital equivalent to those reached in the present experiments are sufficient to keep an animal, if left alone, unconscious for 5 h or more after administration has ceased. The cats were artificially ventilated when spontaneous breathing was

deemed insufficient, while heating devices connected to a computerized feedback system supported temperature regulation. A continuous infusion of 5% glucose in Ringer–acetate solution (pH 7.0) was given to compensate for water and mineral losses. Wounds were infiltrated with lignocaine (5%) to avoid undesirable nociceptive stimulation.

During receptive field mapping, noxious stimulation of the skin evoked discrete and brief contractions of limb muscles, causing fine visible movements. Muscle activity and withdrawal movements ceased when the stimulus was removed. The effects of the noxious stimulation used on blood pressure stability were small or absent. The stimulation did not evoke persistent pupillary dilatation. Typically, each preparation was maintained for ~15 h, which included ~2 h of surgery. At the end of each experiment the animals were killed by an overdose of barbiturate.

### Recordings and stimulation

Muscle activity evoked by calibrated noxious pinch of the skin (pair of forceps with flattened tips; each tip 1 mm<sup>2</sup>) was recorded using an electromyographic (EMG) technique. A small incision was made in the skin overlying the muscle bellies and a reference electrode was inserted subcutaneously at the margin of each wound. Fine steel needle electrodes were inserted into the middle portions of the muscle bellies. Routinely, between two and four muscles were investigated simultaneously. The identity of each muscle was determined by observing the movements evoked by a short train of cathodal pulses (100 Hz, 200  $\mu$ s, 20  $\mu$ A, 20 pulses) through the EMG electrode. The evoked muscle twitches resulted in discrete limb movements. To approximate withdrawal effects on the skin resulting from contractions of each muscle studied, the paw was held against a supporting surface during muscle stimulation, mimicking the action of the muscle when the limb is in a standing position (Levinsson *et al.* 1999).

The hair on the forelimb was trimmed in order to facilitate mapping of cutaneous receptive fields. Noxious stimulation was applied using a pair of force transducer-equipped forceps with flattened tips and the pinch force was maintained at a steady level of 4 N mm<sup>-2</sup>, which caused weak to moderate pain when applied to the skin of our own hands. In order to average out short-term fluctuations of reflex excitability, data were collected during three pinches of each stimulation site (3  $\times$  5 s, interval about 2 s). Data collection started when the pinch force had reached the intended level. The EMG recordings were amplified and high-pass filtered (50 Hz). Motor unit spikes (> 100  $\mu$ V) were counted on-line by computer software (Spike2; Cambridge Electronic Design Ltd, Cambridge, UK) and raw data were stored on hard disc.

### Quantitative analyses

Receptive fields of individual muscles were determined by summation, for each stimulus site, of the number of motor unit spikes evoked during all three consecutive pinches. Using the spike numbers, assigned to their respective stimulus sites (each corresponding to an  $x$ – $y$  coordinate on the forelimb), a high-density grid (~2000 standard points in the  $x$ – $y$  coordinate system) of evoked responses ( $z$  values of the  $x$ – $y$  coordinates) was mathematically derived (Kriging algorithm; Surfer, Golden Software, Inc., Golden, CO, USA). In essence,  $z$  values for a total of 2000 grid points were extrapolated from the spike number values of the original (~33, see Results) stimulation sites. The grid  $z$  values were spatially averaged ('smoothed') to further reduce data noise. Finally, the smoothed grid was used to generate an iso-response map corresponding to a quantitative representation of the spatial distribution of sensitivity within the receptive field.

This quantitative mapping procedure as a whole has been extensively used in previous studies from our laboratory, reliably yielding highly reproducible receptive fields (cf. Petersson *et al.* 2001).

Quantitative receptive fields of climbing fibres were produced in the same way as described above for receptive fields of single muscles. For details on mapping procedures, see Ekerot *et al.* (1991a). In the deeply barbiturate-anaesthetized (40 mg kg<sup>-1</sup> i.p.) preparation the cerebellar anterior lobe was exposed following craniotomy and resection of the occipital lobe. Supplementary doses of the anaesthetic were given i.v. as required to maintain complete muscle atonia, constricted pupils and a stable blood pressure, which remained constant during noxious stimulation of the skin. Activity in single climbing fibres was recorded as complex spikes in the Purkinje cell layer, using a glass coated tungsten microelectrode (exposed tip 10–15  $\mu$ m). The electrical activity was monitored visually on an oscilloscope, and acoustically via a pair of loudspeakers. Climbing fibre responses evoked upon a standard force mechanical noxious pinch of the skin (a clip exerting ~9 N on a skin area of ~2 mm<sup>2</sup>) during a five second period were stored for quantitative off-line analysis. On average, 27 different skin sites were stimulated per receptive field. Stimulation of the most responsive sites yielded tonic activity with average frequencies up to ~10 Hz (a remarkably strong activation of climbing fibres). Typically, the relative extent and location of the receptive field focus was highly consistent between repeated mappings and not influenced by variations in excitability that sometimes affected the absolute size of receptive fields (Ekerot *et al.* 1991a).

In the analysis below, receptive fields of withdrawal reflexes and climbing fibres were compared by linear correlation analysis between ( $z$  values of) the two populations of individual grid points in the high density ( $x$ – $y$ ) grids of evoked responses (cf. Fig. 2) described above. Such a comparison was possible since individual grid points representing withdrawal reflex and climbing fibre receptive fields had the same set of ( $x$ – $y$ ) coordinates. Data for individual comparisons included all grid points falling within any of the two receptive fields to be compared (Schouenborg & Weng, 1994).

The analysis of convergence patterns between cutaneous and muscle afferent input to cerebellar climbing fibres (Fig. 3) was based on quantitative data from a database obtained in a previous study (Jörntell *et al.* 1996). In addition to its cutaneous input, each climbing fibre received mainly group II muscle afferent input from a specific set of muscles (the following were investigated: mm. triceps brachii caput laterale (TRI), biceps brachii caput longum, extensor digitorum communis, extensor carpi radialis longus (ECRL), extensor carpi radialis brevis (ECRB), extensor indicis/propius, extensor digitorum lateralis, extensor carpi ulnaris (ECU), palmaris longus (PL), flexor carpi radialis (FCR) and flexor digitorum profundus (FDP). Typically, individual climbing fibres received input from several synergistic muscles, such as PL, FCR and FDP. In addition it was not uncommon that these inputs also converged with input from muscles acting on a different (set of) joint(s), such as the biceps. For a given climbing fibre, the input strength from each muscle varied in a characteristic way and was quantified by calculating the mean probability of evoking a climbing fibre response with 20 consecutive electrical stimuli to the muscle nerve.

The five climbing fibre units receiving the strongest muscle afferent input from each of the respective muscles were selected



for the present analysis. Outlines of the foci of the cutaneous receptive fields of these five units (see example in Fig. 3A) were then digitized onto a grid representation of the limb ( $\sim 130$  points on the ventral/palmar surface). The contribution of each receptive field focus to a given grid point was weighted according to the magnitude of muscle afferent input to the climbing fibre unit (shown in Fig. 3A as a percentage of maximum response

probability, i.e. 20 out of 20 responses). Following normalization with respect to the maximum value in the grid, the maps were processed in the same way as those of receptive fields of single muscles (cf. Fig. 2A).

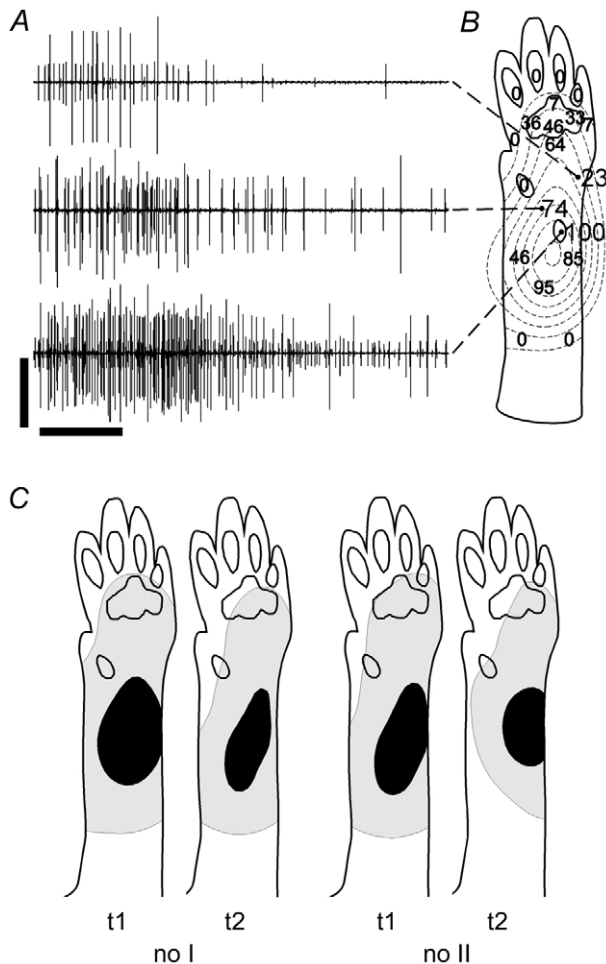
## RESULTS

The cutaneous nociceptive receptive fields of mm. interossei II–V (6 maps, 4 animals), extensor carpi radialis longus (4 maps, 3 animals), extensor carpi ulnaris (4 maps, 3 animals), flexor carpi radialis (2 maps, 1 animal), flexor digitorum profundus (5 maps, 2 animals), palmaris longus (3 maps, 2 animals), flexor carpi ulnaris (4 maps, 2 animals), brachialis (2 maps, 1 animal) and triceps brachii (1 map, 1 animal) in the cat forelimb were determined (Reighard & Jennings, 1966). A pilot survey of some muscle receptive fields has been presented in the context of an investigation addressing spinal cord reflex organization (Levinsson *et al.* 1999).

### Spatial characteristics of cutaneous nociceptive receptive fields

The muscles investigated were reliably activated by noxious stimulation of the skin. Magnitudes of evoked responses were graded with respect to location within the receptive field (Fig. 1A and B), making it possible to obtain quantitative receptive field maps. For each map an average of 33 (range 17–40) standard sites distributed on the dorsal and ventral sides of the forelimb were stimulated in a random order using a standardized pinch force ( $4 \text{ N mm}^{-2}$ ). By counting the total number of unitary spikes evoked during a standard stimulus period for each stimulus site, iso-response levels could be mathematically derived (Fig. 1B; see Methods).

Each muscle had a specific cutaneous receptive field with highly characteristic response topography, i.e. gradients of sensitivity. This conspicuous spatial organization was consistent between consecutive mappings in the same animal and between mappings in different animals (Fig. 1C). A sample receptive field representative of each of the investigated muscles is shown in Fig. 2A. In brief, the interossei (IO), the palmar flexors (FCR, FDP, PL, FCU) and m. brachialis (BRA) all had receptive fields extending exclusively on the ventral aspect of the limb whereas the dorsiflexors (ECRL, ECU) were, to a lesser extent, also activated from the dorsum of the paw and the digits (not shown). The more proximal receptive field of m. triceps brachii (TRI) extended equally on the dorsal and ventral side of the limb and had a focus along the ulnar edge of the forearm close to the elbow. For a given muscle, the extent of the receptive field corresponded to the skin area withdrawn upon contraction of the muscle itself when the limb was in a standing position. The receptive field focus coincided with the patch of skin located where the withdrawal had its maximal amplitude (Levinsson *et al.* 1999).



**Figure 1. Quantification of EMG responses to standardized noxious stimulation allows construction of highly reproducible quantitative receptive field maps**

A, samples of EMG activity evoked during stimulation of cutaneous sites with different sensitivity. Spontaneous activity was zero. Calibration bars: 1 s and 0.5 mV. B, sample receptive field map of m. flexor carpi ulnaris on the palmar side of the left forelimb. Figures indicate response magnitude as a percentage of maximal response. Dashed lines depict mathematically derived iso-response levels (see Methods). C, sample receptive fields of m. flexor carpi ulnaris obtained in two different animals (left and right pair) on two different occasions (left and right in each pair; time intervals of mapping onset were approximately 150 and 220 min, respectively). The smaller size of the second receptive field in each pair (mapped later in the experiment) is coincidental and does not represent a systematic bias in the material as a whole. Grey and black indicate skin areas from which evoked responses were, respectively 0–70 % and 70–100 % (receptive field focus) of maximal response. Left-most receptive field constructed from iso-response level map in B.

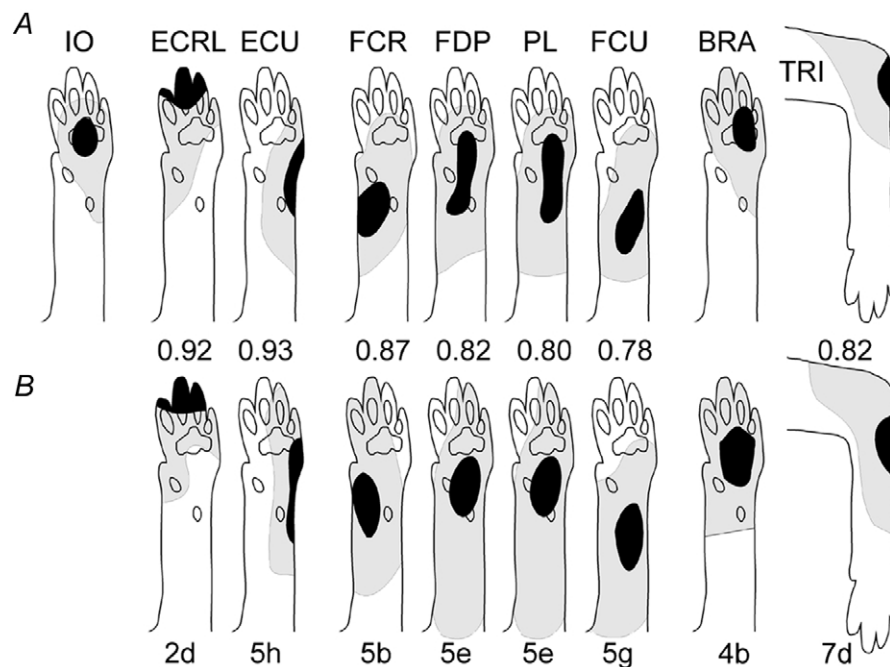
### Comparison between receptive fields of forelimb muscles and climbing fibres

In Fig. 2*B*, quantitatively determined nociceptive fields typical of different subclasses of climbing fibres projecting to the cerebellar C3 zone are shown for comparison (Ekerot *et al.* 1991*a*; Jörntell *et al.* 1996). The visual impression of similarity between receptive fields of specific muscles (Fig. 2*A*) and specific climbing fibre subclasses (Fig. 2*B*) was strongly supported by quantitative analyses, as outlined below. The only muscle receptive field without a climbing fibre receptive field to match was that of the interossei muscles, while receptive fields of (the functionally closely related) *m. palmaris longus* and *m. flexor digitorum profundus* were both similar to the same subclass of climbing fibre receptive fields.

Note that the receptive fields in Fig. 2*B* are representative of a classification system that is based on a total sample of more than 500 climbing fibres mapped in a series of studies (Ekerot *et al.* 1991*a,b*; Jörntell *et al.* 1996; Garwicz *et al.* 1998). Close to 100 of these receptive fields were characterized with methods analogous to those used in the present investigation, thus making quantitative comparison

possible. Such a comparison was carried out here using the mathematically derived evoked response grids from the two sets of data for correlation analysis (see Methods). The results firmly underscore the rather striking similarities between cutaneous nociceptive input to single muscles and to individual climbing fibres with regard to spatial distributions of sensitivity within receptive fields. As indicated between the top and bottom panels in Fig. 2, coefficients of correlation between receptive fields of single muscles and climbing fibres judged to be the 'most similar' ranged from 0.78 to 0.93, with an average of 0.85. By contrast, comparisons between single muscle receptive fields and the 'second most similar' climbing fibre receptive field yielded correlation coefficient values with an average of 0.65 (range 0.21–0.81).

In addition to the above correlation analysis, and based on the same data, the overlap of receptive field foci (> 70 % of maximal responses, as indicated by black in Fig. 2) was calculated as a measure of the similarity between receptive fields of single muscles and climbing fibres. Overlap was defined here as the percentage of common area relative to the area of the smaller receptive field focus. This analysis



**Figure 2. Comparison between receptive fields of forelimb muscles and of climbing fibres reveals high degree of similarity**

*A*, sample nociceptive receptive fields of individual forelimb muscles depicted on the left forelimb. Procedure and conventions as in Fig. 1*C*. IO: *m. interosseus IV*; ECRL: *m. extensor carpi radialis longus*; ECU: *m. extensor carpi ulnaris*; FCR: *m. flexor carpi radialis*; FDP: *m. flexor digitorum profundus*; PL: *palmaris longus*; FCU: *m. flexor carpi ulnaris*; BRA: *m. brachialis*; TRI: *m. triceps brachii (caput longum)*. *B*, quantitatively determined cutaneous nociceptive receptive fields of climbing fibres belonging to the subclasses indicated below each illustration (Ekerot *et al.* 1991*a*). Conventions as in Fig. 1*C*. Receptive fields were mapped in animals under barbiturate anaesthesia. Correlation coefficients obtained by quantitative analysis of the similarity between receptive fields of single muscles and individual climbing fibres (see Methods) are displayed between the two panels. All correlations were statistically highly significant (Student's *t* test;  $P < 0.001$ ). No quantitative data were available for climbing fibre subclass 4b.

showed that overlap between receptive field foci of single muscles and climbing fibres judged to be the 'most similar' ranged from 51 % to 89 % (with the exception of triceps vs. subclass 7d, which was only 32 %), with an average of 72 %. By stark contrast, the overlap between single muscle receptive field foci and the 'second most similar' climbing fibre receptive field focus was on average 17 % (ranging from 0 % to 31 %).

### Convergence between cutaneous and muscle afferent input to climbing fibres

Since a given climbing fibre apparently has a receptive field that is similar to the skin area affected by the action of a single

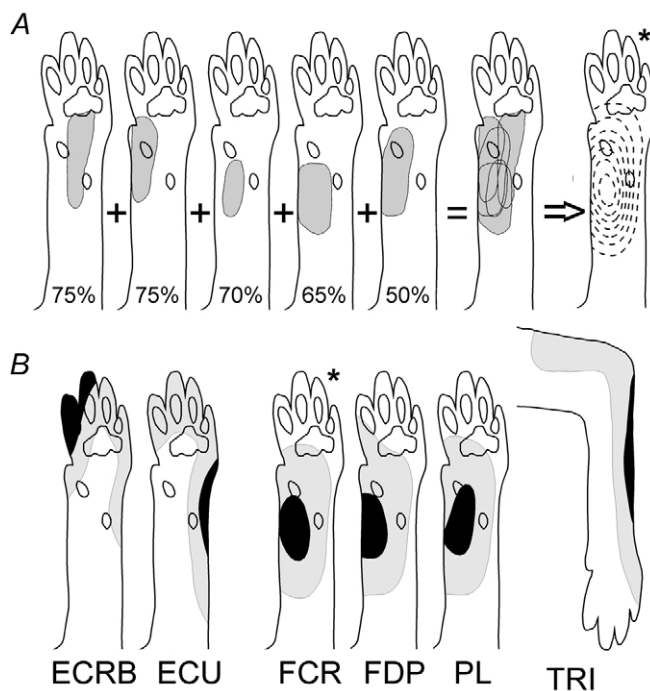
muscle, it was of interest to characterize the relationship between that muscle and the muscle(s) providing group II afferent input to the climbing fibre. In effect, this required a re-assessment of the patterns of convergence between cutaneous and muscle afferent input to climbing fibres. There is no simple one-to-one relationship between the two types of input. Afferents from several muscles (including not only synergists but in some cases also those acting across different joints) often converge on individual olivary neurons and, partly as a consequence of this, our previous analysis of convergence patterns between muscle input and skin input yielded rather complex results. Crucially, the functional relationship between skin and muscle afferents converging upon single olivary neurons was interpreted with reference to the biomechanics of a free limb position (Jörntell *et al.* 1996). By contrast, the above findings suggest that the input to paravermal climbing fibres, by analogy with the withdrawal reflexes, is functionally adapted to a standing limb position (Schouenborg & Weng, 1994; Levinsson *et al.* 1999).

Figure 3A shows the principles of the present analysis. The resulting patterns of convergence between cutaneous and muscle afferent input are illustrated in Fig. 3B. From a sample of on average 52 climbing fibres tested for input from each of the muscles indicated, the five climbing fibres receiving the strongest afferent input from each muscle were selected, but using any number between three and ten climbing fibres yielded similar results. The foci of the selected cutaneous receptive fields were superimposed on outlines of the limb and the cutaneous areas derived from averaging the superimposed foci (cf. Fig. 3A) are shown.

As seen by comparing Figs 3B and 2A, the derived cutaneous areas and the withdrawal reflex receptive fields of the corresponding muscles display suggestive similarities. Although these similarities are not as striking as those between receptive fields of single muscles and individual climbing fibres (Fig. 2A and B, respectively), the relationship is nevertheless readily interpretable within the framework of withdrawal reflex function. These findings indicate that the olivary target of group II muscle afferents from a given muscle is mainly the climbing fibre subclass with a cutaneous receptive field similar to that of the muscle itself. Afferents also target climbing fibres in other subclasses, but to a lesser extent. The functional significance of these patterns of convergence between muscle input and skin input will be discussed below.

## DISCUSSION

The present study demonstrates that with regard to spatial characteristics, nociceptive receptive fields of individual forelimb muscles and receptive fields of climbing fibres projecting to the cerebellar C3 zone are similar. This finding is strongly supported by the quantitative measures



**Figure 3. Patterns of convergence between cutaneous and muscle afferent input to climbing fibres reveal specific relationship to withdrawal reflexes**

A, superimposition (cf. Asanuma *et al.* 1968) and averaging of cutaneous receptive field foci. Five climbing fibres with different receptive field foci and strong muscle afferent input from m. flexor carpi radialis are shown as an example. Percentages indicate relative strength of muscle afferent input (probability of occurrence of a climbing fibre response upon stimulation of the muscle nerve; for details see Jörntell *et al.* 1996), which determined the relative contribution of each receptive field focus to the average receptive field map to the right (see Methods). Dashed lines indicate iso-response levels. B, averaged maps of climbing fibre receptive field foci ( $n = 5$ ) associated with input from the muscles indicated. Abbreviations: cf. Fig. 2A; ECRB: m. extensor carpi radialis brevis. Based on functional similarity, this muscle is shown instead of ECRL, which provides only weak input to climbing fibres (Jörntell *et al.* 1996). Muscle afferent input from mm. interossei, flexor carpi ulnaris and brachialis was not assessed. The overlap between the centre areas (black) of these 'convergence maps' and receptive field foci of the corresponding muscles in Fig. 2A was on average ~50 %, ranging from approximately 10 % to 90 %.



of similarity provided by correlation analysis and overlap analysis of receptive field foci. In addition, a novel analysis of convergence patterns between cutaneous and group II muscle afferent input to individual climbing fibres indicates that afferents from a given muscle preferentially target climbing fibres with cutaneous receptive fields similar to that of the muscle itself. It should be emphasized, however, that the muscle afferent–climbing fibre projection is characterized by both divergence (from any given muscle) and convergence (on any given climbing fibre).

Both climbing fibres and withdrawal reflexes display cutaneous receptive fields with detailed spatial distributions of sensitivity. We therefore argue that the high degree of similarity between the two types of maps must indicate that the encoding of sensory information is indeed equivalent in the two systems. Furthermore, it is highly unlikely that the functionally straightforward relationship between cutaneous and muscle afferent input to individual climbing fibres is coincidental. We interpret this as additional support for a functional link between muscle action and salient features of the input to individual climbing fibres.

Note in this context that the notion that cerebellar afferent systems convey information that is not of a simple sensory nature but instead reflects parameters of motor output has been previously advanced and documented for mossy fibre afferents by Bosco & Poppele (2000) and Bosco *et al.* (2000). Their findings indicate that the activity of certain individual neurons in the dorsal spino-cerebellar tract is related to limb kinematics resulting from muscle activation rather than to the tension developed by individual muscles.

### Further similarities and their functional implications

The similarity between the two types of cutaneous receptive fields is not the only characteristic shared by this portion of the climbing fibre system and the withdrawal reflex system. Both systems have modular organization and comparable numbers of modules (in the rat hindlimb, most muscles have been investigated; see Reighard & Jennings, 1966); both receive convergent multi-modal input from the skin (Ekerot *et al.* 1987a; Weng & Schouenborg, 1998) and are strongly activated by nociceptive cutaneous input (Ekerot *et al.* 1987b; Ekerot *et al.* 1991a; Garwicz *et al.* 1992). A straightforward explanation for these indications of functional relatedness is that the inferior olivary neurons receive their input from a spino-olivary pathway that originates in axon collaterals of interneurons intercalated in spinal nociceptive withdrawal reflex modules (Ekerot *et al.* 1991b). However, since the present investigation does not specifically address whether or not there is an anatomical connection between these particular reflex modules and climbing fibres we refrain from drawing conclusions relating to this issue. Nevertheless, the parallels demonstrated between with-

drawal reflex function and the patterns of input to cerebellar climbing fibres are worth exploring.

Hence, we suggest that the analogous encoding of cutaneous information in the two systems implies that climbing fibres to a given paravermal module are well suited to detect sensory events reflecting the activity of a single muscle. Consider the fact that the spatial distribution of sensitivity within a cutaneous climbing fibre receptive field is directly proportional to the spatial distribution of withdrawal efficacy of a particular muscle. This means that the probability that a given climbing fibre receptive field will encounter an external object and thus be activated at any point in time will be inversely proportional to the level of contraction of that muscle. Moreover, once the receptive field does encounter an object, the degree of nociceptive activation of the climbing fibre will be in proportion to the extent to which the muscle has the capacity to influence (withdraw) the specific patch of skin that has been stimulated. An important consequence of such encoding of sensory input is that afferent information is represented in a way that is directly relevant to motor corrective signals or training signals in the context of sensory guidance of movements or movement error detection in motor learning (Wolpert *et al.* 1998). This would effectively facilitate the computation of neuronal networks performing sensorimotor transformations in such contexts and thus automatically allow the acquisition of sensory data in a form relevant to motor function (cf. Bower, 1997).

Adding to the intricacy of the climbing fibre system, the same muscle that influences a particular skin surface is also a major source of group II muscle afferent input to olivary neurons with receptive fields corresponding to that surface. To a lesser extent, afferents from this muscle also target climbing fibres of 'neighbouring' receptive field subclasses. Exploring the parallelism with input–output relationships of withdrawal reflexes, the highly convergent input to paravermal climbing fibres may therefore be assumed to represent (i) cutaneous input to an individual spinal withdrawal reflex module, (ii) muscle afferent input from the muscle that constitutes the output from that module and (iii) muscle afferent input from muscles that constitute the output from functionally related modules. This suggests that an individual climbing fibre signals cutaneous sensory events reflecting activity of a single muscle conditional upon the functional state of the muscle itself and of other, functionally related, muscles. Hypothetically, this organization may provide a mechanism for facilitating or suppressing transmission of cutaneous input ('gating'; Apps 1999) that is not directly dependent on descending motor commands.

In real life motor behaviour, single muscles rarely – or never – act in isolation. Instead, their actions constitute



building blocks of compound, multi-joint movements or postures. The modular organization of paravermal networks endows the cerebellum with the capacity to parallel-process sensory events correlated to individual movement components or postural components by allowing independent use of afferent signals related to the action of individual muscles. By contrast, the output from most individual modules of nucleus interpositus anterior appears to influence several muscles which act as a group on two or three joints of the forelimb (Ekerot *et al.* 1995; see also Fetz *et al.* 1989; Belhaj-Saif *et al.* 1998; McKiernan *et al.* 2000). One may assume that this 'discrepancy' between an input reflecting single muscle action and a divergent multi-joint/multi-muscle output is an important clue as to how these cerebellar modules operate.

### Cerebellar monitoring of spinal reflex pathways

Extrapolating the findings of an 'action-based' organization of input to climbing fibres in the paravermal cerebellum, we propose that other cerebellar afferent representations of the body may also be related to afferent representations in spinal or brainstem reflex systems. The large, sometimes discontinuous receptive fields that characterize certain portions of the olivo-cerebellar climbing fibre system may reflect afferent input to spinal reflex circuits (or spinal pattern generators) acting on larger and/or disjunctive groups of muscles (Garwicz *et al.* 1992). Relevant in this context is the role of climbing fibres in classical conditioning of the eye-blink reflex, a paradigm exploring cerebellar involvement in motor learning (Yeo & Hesslow, 1998). The possibility that climbing fibres may indeed be 'monitoring' afferent input to the brainstem reflex pathway mediating the eye-blink response strongly supports the hypothesis that, in classical conditioning, climbing fibres convey information about the unconditioned stimulus to the cerebellar cortex. Evidence for this assumption has to date been inconclusive.

### Is this representation of the body surface 'somatotopic'?

Notably, a muscle action related representation of the skin, such as that proposed above, would be, in some sense, inherently modular. Changes in limb movement or posture caused by contractions of single or small groups of muscles do not form a continuum with respect to areas of skin being displaced. A sum total of the skin areas displaced upon contraction of single or small groups of muscles would therefore give rise to a fragmented representation of limb/body surface (see Kaas, 1997). If this organizational principle applies to the peripheral climbing fibre input to the cerebellar paravermis, one may question whether this should be regarded as a map of the body (or body surface) *per se*. Rather, the governing principle of the representation should be its close relationship to a particular characteristic of the motor plant, namely the action of its smallest units, the single muscles.

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### Acknowledgements

Supported by the Swedish Medical Research Council Projects No. 1013 and 14015, the Medical Faculty of Lund University, Astra Hässle Inc., Knut and Alice Wallenberg's Foundation, Elsa and Thorsten Segerfalk's Foundation, Greta and Johan Kock's Foundation, the Crafoordska Foundation, Magn. Bergvall Foundation, Swedish Society for Medical Research and The Royal Physiographic Society in Lund.