ISAS - INTERNATIONAL SCHOOL FOR ADVANCED STUDIES

"Parallel processing in the leech central nervous system"

Thesis submitted for the degree of "Doctor Philosophiae"

CANDIDATE
Giulietta Pinato

SUPERVISOR
Prof. Vincent Torre

SISSA - SCUOLA INTERNAZIONALE SUPERIORE DI STUDI AVANZATI

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To my mother

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Note

The work described in this dissertation was carried out at the International School for Advanced Studies, Trieste, between November 1996 and December 1999. All work reported arises solely from my own experiments and has not been submitted, as in whole or in part, to any other University.

Giulietta Pinato

Abbreviations used in the text

AC: anterior connective

AE: annulus erector motor neuron

AGAR: anterior ganglion, anterior root

AGPR: anterior ganglion, posterior root

AP: anterior pagoda cell

CGAR: central ganglion, anterior root

CGPR: central ganglion, posterior root

CNS: central nervous system

CV: coefficient of variation

DE: dorsal excitatory motor neuron

EPSP: excitatory post synaptic potential

LAR: left anterior root

LDPR: left dorsal-posterior root

LPPR: left posterior-posterior root

LPR: left posterior root

N_l: lateral nociceptive neuron

N_m: medial nociceptive neuron

PC: posterior connective

P_d: dorsal pressure mechanosensory neuron

PGAR: posterior ganglion, anterior root

PGPR: posterior ganglion, posterior root

P_v: ventral pressure mechanosensory neuron

RAR: right anterior root

RDPR: right dorsal-posterior root

RPPR: right posterior-posterior root

RPR: right posterior root

T_d: dorsal touch mechanosensory neuron

T_l: lateral touch mechanosensory neuron

T_v: ventral touch mechanosensory neuron

VI: ventral inhibitor motor neuron

Abstract

The aim of this thesis is to shed light on the parallel processing in the leech nervous system. Two main problems have been addressed:

1) how a mechanical stimulus is coded by mechanosensory neurons at the first stage of the information processing in the central nervous system (CNS).
2) which statistical properties of the electrical activity of neurons in the segmental ganglion are relevant for information processing and in particular for motor reactions.

A semi-intact preparation consisting of a single segment innervated by its own ganglion and the adjacent ganglia was used. Sensory stimulation was delivered by touching the skin with an appropriate device. Previous observations indicate that each point of the skin surface is innervated by several mechanosensory neurons (Nicholls and Baylor, 1968; Yau, 1976; Lewis and Kristan, 1998-c). In my thesis I analyzed how sensory information is coded in the electrical activity of a population of mechanosensory neurons. I developed and used an experimental set-up allowing the recording of the simultaneous activation of all mechanosensory neurons belonging to three consecutive segmental ganglia. The experiments were performed in high Mg^{2+} Ringer fluid in order to block all chemical synapses so as to eliminate synaptic responses and muscle contraction leading to a motion of the skin resulting in an additional sensory feedback.

In these conditions it is possible isolate the responses of sensory neurons directly activated by the stimulus. The extent of the activation of mechanosensory neurons in three adjacent ganglia after a stimulation was analyzed. The results of this mapping show that all mechanosensory neurons are consistently activated also in their accessory receptive fields. The reproducibility of their response is the same as that observed in mechanosensory neurons responding in major receptive fields. T cells activation presents the same characteristics for stimulation of the major or minor receptive fields, indeed they respond to the transient phase of the stimulus. P cells show a different dynamics of activation if stimulated in their major or minor receptive field. In presence of a prolonged stimulation, P cells of the central ganglion fire action potentials continuously while P cells of accessory ganglia adapt. N cells are always activated in major and minor receptive fields showing approximately the same dynamics. When the skin is touched with a moderate tactile stimulus, i.e. exerting a force of less than 20 mN, many different neurons (T and P cells) of the three ganglia fire action potentials. Thus sensory coding is initially redundant. If the stimulation is prolonged, many of these sensory neurons rapidly adapt and only P cells of the central ganglion respond. Thus sensory coding is dynamical and becomes very sharply tuned.

In the second part of my PhD work, a different stage of the information flow has been investigated. The aim of the research was to study which statistical properties of the electrical activity of neurons in the leech ganglion are important for neural processing. In particular I analyzed the statistics of the evoked activity in response to a sensory input. The ganglion was considered as a black box with inputs and outputs; inputs are represented by sensory neurons and outputs by motor neurons or other neurons

innervating roots and connectives. The responses of mechanosensory neurons to touch stimuli show trial-to-trial reproducibility, i.e. spikes in sensory neurons occur at very precise times. This does not happen for motor neurons. The experiment consisted in stimulating repeatedly a particular mechanosensory neuron by evoking action potentials with an intracellular electrode. The evoked activity in response to this input was recorded by suction electrodes. The extracellular recordings contain the superimposed activity of many neurons which can be separated by spike sorting procedure. The first and second order statistics were studied. Two main results were obtained by this analysis:

1) the response is distributed on many neurons; many neurons respond to the same stimulus and the same neurons are recruited for different stimuli.

2) the response is characterized by spatio-temporal variability, i.e. different neurons respond in different trials and neurons respond with jitter in time occurrences of spikes.

The comparison between reproducibility of mechanosensory neurons evoked by touch stimulus and motor neurons evoked by mechanosensory neurons activation shows that sensory cells are very reproducible, therefore the origin of motor neuron variability has to be found in synaptic transmission. The analysis of the second order statistics was used to check correlation among coactivated neurons. Joint entropies and joint probabilities for each pair of neurons were computed and compared with the sum of individual entropies and the product of individual probabilities, respectively. In all cases these quantities are consistently equal. This means that coactivated neurons are statistically independent. This property of the system can be functional for neural processing. If the response of the network is distributed, then the pooling of the electrical activity of all individual responses is important and may be the key feature of

the network. In this perspective, a distributed process consisting in a large number of statistically independent unreliable elements leads to a reproducible response, when the response is pooled over all elements. There is theoretical justification for this statement, indeed it is demonstrated that the pooling of a large number of statistical independent stochastic processes affected by high variability lead to a stochastic process with low variability. In conclusion, increasing the number of elements pooled, the response is more and more reliable.

1 Introduction and background

A major issue of contemporary neuroscience is to understand how information is processed in the nervous system, i.e. how the nervous system is capable to interact with the external environment by producing behavioral outputs in response to various kinds of sensory inputs.

In this thesis some aspects of the parallel processing were addressed in the simple nervous system of the leech *Hirudo Medicinalis*. Following the direction of the information flow, the coding of touch stimulation by a population of mechanosensory neurons was initially investigated. Then the statistical properties of motor neurons activated by sensory stimulation were analyzed.

In this first chapter I will introduce the topics of my thesis by summarizing the main results previously obtained. Some basic information about the neurobiology of the leech are summarized in section 1.1; this will be useful for understanding the methodological approach to the issues I have addressed in my thesis. The most important publications on the sensory coding in the leech are reviewed in section 1.2. In section 1.3 the issue of variability of the electrical activity in the nervous system is reviewed. This section provides the background and rationale for my work. The last section of this chapter (1.4) describes the aim of my thesis.

1.1 The nervous system of the leech

1.1.1 General properties

Several classic problems of neuroscience have been addressed in the leech CNS such as the development, regeneration and repair (Weisblat and Shankland, 1985; Shankland, 1995; Jellies and Johansen, 1995), the neuronal basis of behavior, including the study of modulation, integration, coordination of the activity of many neurons (Stent et al.1978; Friesen et al. 1976, Shaw and Kristan, 1997), the neuronal basis of learning (Boulis and Sahley, 1988; Sahley, 1995; Modney et al., 1997), the study of the impulse diffusion in the branching neurons (Muller and Scott, 1981; Macagno et al. 1987; Gu, 1991; Gu et al., 1991; Melinek and Muller, 1996; Baccus, 1998).

The reason why these specific questions have been addressed in the nervous system of the leech are both historical and related to the system itself. As will become clearer in the following pages, this preparation presents a very schematic structure with the possibility to investigate the role of identified neurons, to clearly follow the flow of information in the nervous system, and to monitor the phases of its development and repair. Moreover, the limited number of behaviors supported by the animal allows to describe in detail neuronal circuits underlying them.

Leeches are segmented worms belonging to the class of Hirudinea, which includes about 650 species. The *Hirudo Medicinalis*, which has been used in my experiments and generally is the most studied species of leeches in neurobiology, will be described here.

This animal presents segmentation, i.e. the body is composed of the repetition of a fixed number of similar units which are linked and coordinated one another. The structure of the CNS reflects this segmentation. As illustrated in Fig. 1.1, it consists of a ventral nerve cord composed of a chain of 21 segmental ganglia linked one to another by bundles of axons (connectives). The head and tail regions are more specialized and are innervated by two groups of ganglia originated by the fusion of some segmental ganglia. The segmental ganglia are numbered sequentially from the head region (anterior) to the tail region (posterior).

Each of the segmental ganglia innervates a well defined segment of the body wall by two pairs of nerves (roots) arising symmetrically from the left and right sides. The four roots are defined as anterior and posterior, left and right. The two posterior roots bifurcate near the ganglion, each one originating two branches called posterior-posterior roots and dorsal posterior roots. By these nerves the ganglion innervates the whole segment. The anterior and posterior-posterior roots innervate the territory corresponding to the lateral and ventral part of the animal, while the dorsal posterior roots innervate the dorsal part of the animal.

The major portion of the body wall is made up of three superimposed layers of muscles; from the inner to the outer part of the body are located the longitudinal, oblique and circular muscle fibers respectively. Longitudinal muscle contraction shortens the animal, while circular muscle contraction elongates it. The oblique muscles are responsible for twist movements of the body. Dorso-ventral muscle fibers run between the dorsal and ventral parts of the body wall and their contraction flattens the animal.

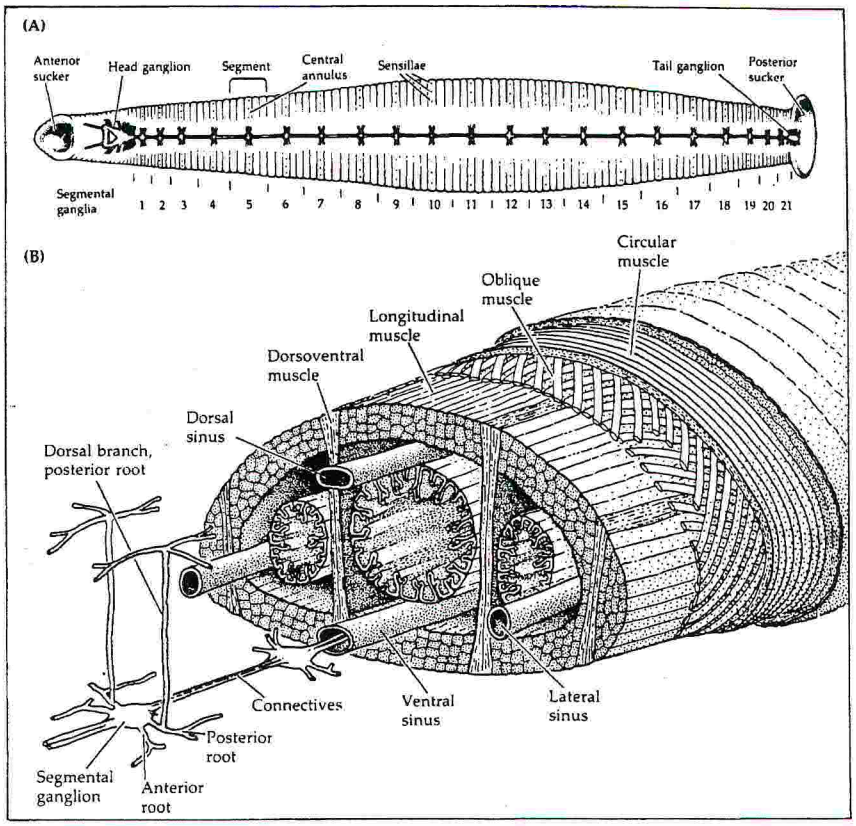

Fig. 1.1 The leech central nervous system. A: Schematic diagram of the leech showing the segmentation and the two specialized regions of anterior and posterior suckers. The CNS consists of a ventral nerve cord composed of a chain of 21 segmental ganglia and two more specialized head and tail ganglia, linked one to the other by bundles of axons (connectives). Segments are indicated, composed of five annuli, the central one of which contains sensillae, specific sensory organs. B: Cross-section of the leech showing its anatomy. The body wall is made-up of three layers of muscles (circular, oblique and longitudinal), dorso-ventral muscle fibers run from the dorsal to the ventral side of the animal. The nerve cord lies in the ventral part of the body and it is surrounded by the ventral blood sinus. Ganglia innervate the body wall through the anterior and posterior roots. The posterior root bifurcates near the ganglion and the dorsal branch crosses ventro-dorsally the body to innervate the dorsal region. The other roots innervate lateral and ventral regions.

(From: Nicholls et al., 1992)

A further kind of muscles are located immediately under the skin and are responsible for erecting annuli in ridges.

The leech segmental ganglion contains the cell bodies of about 400 neurons. Its aspect and structure are conserved from segment to segment and from animal to animal; indeed the same neurons are recurrent in each ganglion for their position, dimension and function. Fig 1.2 is an example of the recurrent position of the three kinds of mechanosensory cells in the segmental ganglion.

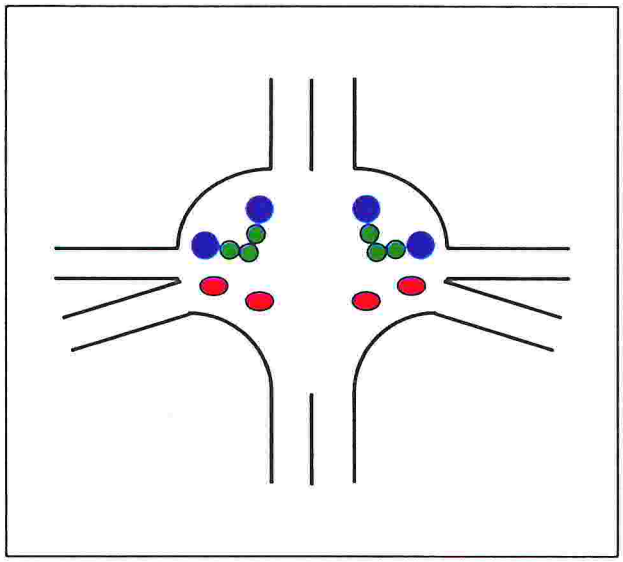

Fig. 1.2 Diagram of the recurrent positions of the different kinds of mechanosensory cells in the leech ganglion. In green are indicated the six T cells, in red the four P cells and in blue the four N cells.

All neurons in the leech ganglion are monopolar; the cell bodies are contained in separated regions (packets) while their innervations take place in the neuropile, a specialized region in which synapses are arranged.

The neurons can be roughly divided into three categories on the basis of their function: sensory neurons, interneurons and motor neurons. Sensory neurons are neurons that directly translate a physical input coming from the environment to the animal in action potentials, or more generally are specialized to transform a physical quantity like light, pressure, concentration etc. into a change of the electrical properties.

The category of interneurons broadly contains all neurons whose entire arborization does not exit from the CNS.

Motor neurons are responsible for the excitation or inhibition of muscles (Stuart, 1970; Mason and Kristan, 1982)

Other excitable cells project their branches outside the CNS, for example Retzius cells whose firing is related to serotonin release (Lent, 1973; Willard, 1981; Mar and Drapeau, 1996) and AP cells (Gu, 1991; Gan and Macagno, 1995; Melinek and Muller, 1996) of still unknown function.

1.1.2 Properties of mechanosensory neurons

The identification of cells responsible for touch was carried out by Nicholls and Baylor (1968), who found three kinds of neurons responsible for three different mechanosensory modalities (see Fig.1.2): the T (touch) cells are three for each hemiganglion and respond to a light mechanical stimulus, the P (pressure) cells are two for each hemiganglion and respond to stronger stimuli, and N (noxious) cells are

two for each hemiganglion and respond to a damaging stimulation of the skin. Recent studies have demonstrated that N cells exhibit also functional properties similar to those of polymodal nociceptive neurons in mammals (Pastor et al., 1996), i.e. they are not simple mechanosensitive cells but respond to different sensory modalities of noxious stimulus like high temperature and irritant chemical substances.

1.1.3 Receptive fields

Receptive fields of mechanosensory neurons have been also investigated (Nicholls and Baylor, 1968). It has been found that sensory cells in each ganglion innervate a well defined territory of the corresponding segment, for example the three T cells respond respectively to the stimulation of the ventral (T_v), lateral (T_l) and dorsal (T_d) region of the segment. Analogously, P cells respond one to ventral (P_v) and the other to dorsal (P_d) touch stimuli. For N cells not well defined receptive fields were found, the receptive fields for the two ipsilateral N cells (N_m N_l) in the same ganglion being approximately coincident (Blackshaw et al., 1982).

From the study of the anatomy and physiology of mechanosensory neurons, their receptive fields appear to be composed of several non overlapping sub-fields (see Fig. 1.3B) innervated by separate branches of the same cell (Nicholls and Baylor, 1968) passing through the roots of their ganglion and through that of adjacent ones (Yau, 1976).

On the other hand, by comparing the extension of receptive fields of cells responsible for the same sensory modality in a single ganglion or in two adjacent ones, a

considerable overlapping between receptive fields has been observed, as shown in Fig. 1.3A (Nicholls and Baylor 1968).

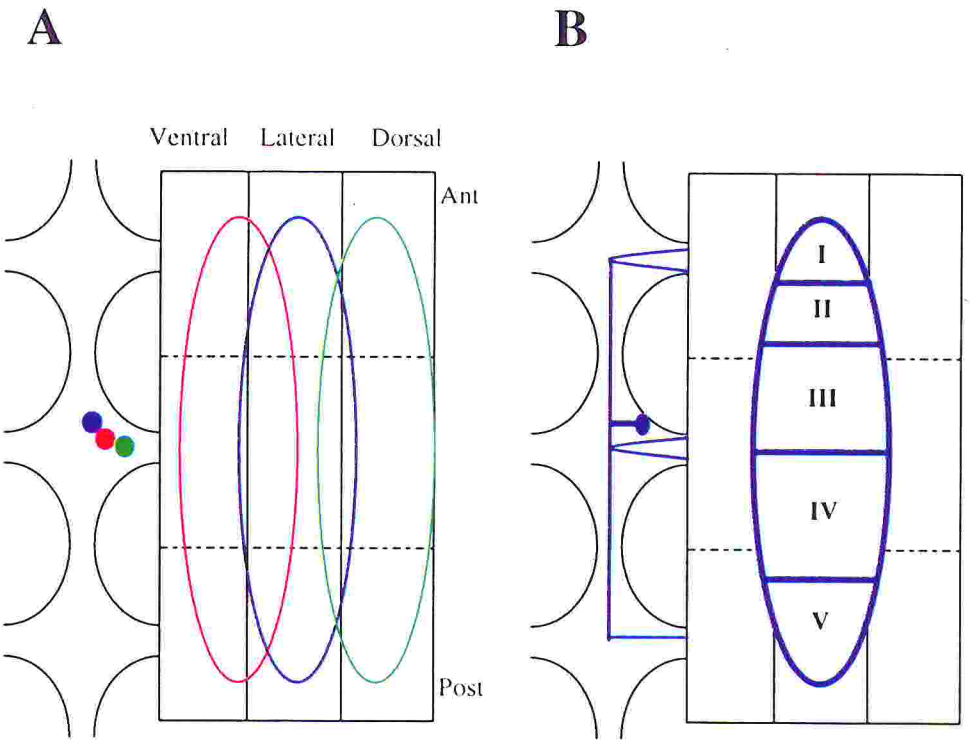

Fig. 1.3 Organization of the receptive fields. A: Boundaries of the receptive fields of three T cells in the same ganglion overlap. The cells are drawn in the same color as their receptive fields. T_v is represented in red, T_l in blue, T_d in green. B: Adjacent subfields (I II III IV V) of a T_l cell, innervated by separated branches, do not overlap

The anatomical organization of axon branches indeed reflects the results obtained for the receptive fields (Muller and Mcmahan, 1976; Blackshaw, 1981, Blackshaw et al., 1982). Mechanosensory neurons are characterized by a main process arising from the

cell body that gives rise to several branches projecting through the ipsilateral roots and the connectives to innervate the roots of adjacent ganglia. The finer processes are located in each ganglion in the region called neuropile, the site in which synapses between neurons are concentrated.

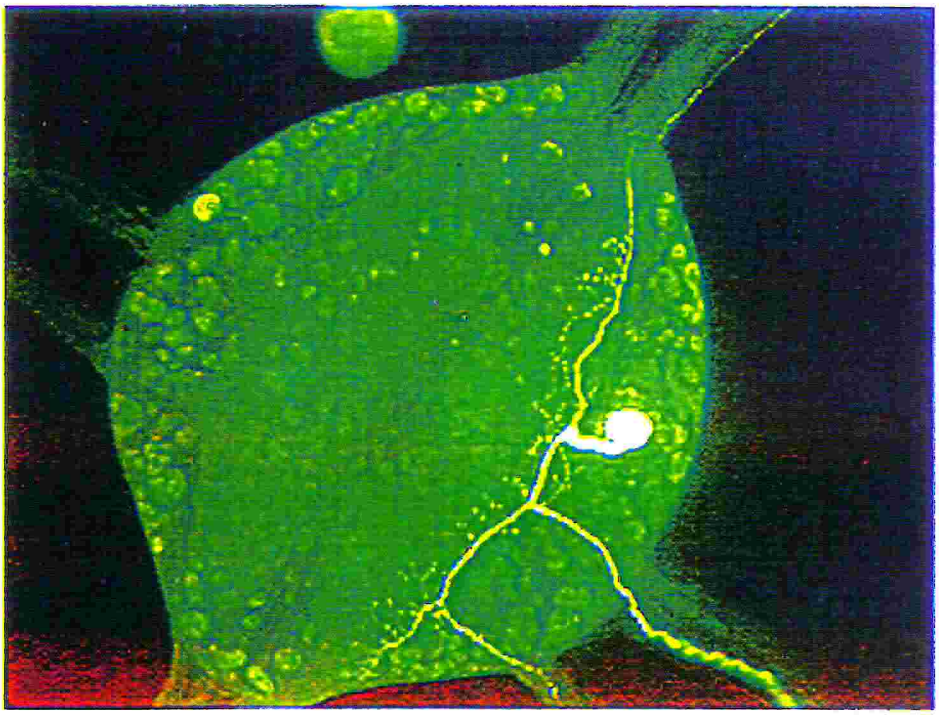

Fig. 1.4 Lucyfer Yellow injection of a T cell, showing the pattern of arborization. Each T cell body gives out a single process which branches in four main processes. Two thick ones going to ipsilateral roots, and two thinner ones going to adjacent ganglia.

Fig. 1.4 shows the pattern of arborization of a T cell, injected with Lucyfer Yellow. In each ganglion T cell bodies give out a single process which branches in four main processes. The thick ones project to ipsilateral roots, while the thinner ones innervate the two connectives.

1.2 Processing of mechanosensory information in the leech CNS

The characterization of neurons responsible for different touch sensory modalities and the mapping of their receptive fields represent the first step for studying sensory coding in the leech CNS. However this is not enough to explain how a complex sensory input is processed. Several mechanisms are involved even in a simple nervous system and their description is necessary to understand basic features of neural processing.

1.2.1 Role of conduction block

The discovery of the presence of major and minor receptive fields for mechanosensory neurons, and the observation that conduction block (Baylor and Nicholls, 1969; Van Essen, 1973; Yau, 1976) takes place in these cells, has been an important input for the investigation of the mechanisms able to modulate the influx of information through the CNS. It was observed that the impulses originating in the minor receptive fields can be blocked in particular sites of the neurons. Conduction block is an activity dependent phenomenon occurring when cells fire for prolonged time. Trains of impulses of a

neuron activate an electrogenic pump generating hyperpolarization that increases the firing threshold. This can lead to the failure of impulse initiation between fine and thicker axons at the branch points, where the disparity in the axons diameters represent an unfavorable condition for transmission (Jansen and Nicholls, 1973; Yau, 1976; Muller and Scott, 1981).

The phenomenon of conduction block has been deeply investigated in order to understand a possible functional role.

A first interpretation was related to the fact that conduction block isolates major receptive fields, since information from adjacent segments cannot reach the ganglion. Moreover, because of its activity dependence, it has been thought to act as a low pass filter mechanism for action potentials generated in the minor receptive fields traveling to the CNS (Yau, 1976).

A further investigation led to the observation that failure of action potentials transmission in neurons can affect the synaptic efficacy since parts of the neuron are silenced. In this way the activity can be responsible for switching the pattern of synaptic connections of the neuron from one configuration to another (Muller and Scott, 1981; Macagno et al., 1987; Gu, 1991). As recent studies report, another activity dependent phenomenon related to action potential failure has been observed to influence synaptic efficacy by facilitating synapses: this is the case of reflection (Baccus, 1998).

Neuromodulation of conduction block has also been studied (Mar and Drapeau, 1996), and it has been found in particular that serotonin, as well as activation of Retzius cells, has strong effects on the duration of conduction block.

1.2.2 Behavioral role of mechanosensory neurons

Beside the characterization of physiology and anatomy of mechanosensory neurons described above, other studies have examined the roles of the mechanosensory neurons in relation to the behavior.

The problem of representation of touch by the leech CNS has been addressed in correlation with the behavioral output, since the sensory cells appear to be the major contributor to trigger the most part of motor responses such as local bending (Kristan, 1982), shortening (Kristan et al., 1982) and swimming (Debski and Friesen, 1987).

The particular case of local bending has been thoroughly investigated, and it appears very appropriate to address the problem of sensory location coding. A light mechanical stimulus of the skin produces a local bend in the leech away from the touched site. This reflex involves the contraction of longitudinal muscles on the stimulated side and the relaxation of those in the opposite direction. The activation of different patterns of mechanosensory cells represents the first coding of the stimulus location that is transferred to the inter neurons layer and finally to the motor neurons responsible for motor reflex. The touch location information is therefore represented subsequently by three groups of neurons (Lewis and Kristan, 1998a). The accuracy of the representation carried out by sensory cells has been evaluated on the basis of the analysis of the tuning curves and compared to that achieved by motor neurons as local bending response (Lewis and Kristan, 1998a-b-c). These studies demonstrate how touch location information is processed, either at the input (sensory cells) or at the output (motor neurons), by population coding, i.e. by integrating the activity of many neurons.

The role in behavior of different kinds of mechanosensory neurons has also been studied by looking at the different kinetics of activation and adaptation during natural

stimulation (Carlton and McVean, 1995). These experiments suggest that T cells are responsible for detecting features of mechanical environment, since they respond to the rate of skin indentation. On the other hand P cells show tonic responses to prolonged stimulation of higher threshold, detecting occasional pressure peaks and eventually triggering behavior initiation. Only rarely, in normal environments, are N cells activated.

1.3 Variability and reliability of the nervous system

An intrinsic property of the nervous system seems to be that discharge patterns of action potentials in neurons are affected by a large variability. This has been observed both in mammals and in lower animals. The question arises if neuronal variability represents an obstacle for reliability in nervous system rather than a functional property. In this section some studies in which this issue has been addressed will be reviewed in order to understand its multiple causes.

Information processing and neural computation are performed in the nervous system by exploiting biophysical properties of axons, dendrites and synapses (Koch and Poggio, 1987). Some mechanisms like action potential initiation and conduction (Stuart and Sakmann, 1994), excitatory and inhibitory synapses, depression and facilitation in synaptic transmission (Gerstner et al., 1997) were elucidated in order to understand their role in basic neural operations. In many circumstances it is demonstrated that the nervous system is able to protect information from noise (Laughlin, 1987), it responds reproducibly and encodes external stimuli very accurately (Bialek et al., 1991; Bialek

and Rieke, 1992; De Ruyter van Steveninck and Bialek, 1988; Rieke et al., 1995; Rieke et al., 1997; De Ruyter van Steveninck et al., 1997; Juusola and French, 1997; Abbott and Dayan, 1998). On the other hand, several experiments performed on cortical neurons in vivo (Reich et al., 1997; Gur et al., 1997; Shadlen and Newsome, 1998) and in invertebrates like insects (Warzecha and Egelhaaf, 1999) or mollusks (Wu et al., 1994b) reveal a large variability in spike trains and from observations of synaptic connectivity in hippocampus a significant unreliability emerged in synaptic transmission characterized by high failure rate. Causes of unreliability in the response of neurons have been widely investigated at the level of single unit.

Possible sources of unreliability in transmission at individual synaptic contacts on CA1 hippocampal pyramidal neurons have been evaluated (Allen and Stevens, 1994). Many sources (threshold fluctuation, conduction failures, temperature dependence) were considered, leading to the conclusion that probabilistic release mechanisms at low capacity synapses are mainly responsible for the unreliability of synaptic transmission. Several other studies have focused on mechanisms underlying synaptic transmission linking reliability of synapses to plasticity. In particular release properties of synapses and the anatomy of connectivity between cortical pyramidal neurons have been deeply investigated by Markram et al. (1997), while in papers by Hessler et al, (1993) and Murthy et al. (1997) the probabilities of transmitter release in individual hippocampal synapses are directly measured. The evidence of unreliability at central excitatory synapses is nevertheless controversial, as it arises in very detailed investigations on synaptic transmission variability conducted by Wahl et al. (1995), Larkman et al. (1997) and Hardingham and Larkman (1998).

Many models are proposed to mimic the variability observed in vivo in which the neuron is considered as an input-output element converting spike trains coming from many presynaptic neurons in a output spike train (Shadlen and Newsome, 1998; Softky and Koch,1993). In the work by Mainen and Sejnowski (1995) it is established that intrinsic noise neuron is low. Namely, the mechanisms of transformation of somatic currents in spike trains within a cortical neuron seem very reliable. This finding corroborates the hypothesis that synapses driving neurons, being subject to both release failures and quantal fluctuation, are mainly responsible for variability. In order to test whether the in vivo variability could be due to postsynaptic currents generated by independent synaptic inputs, neurons in vitro can be injected with input tested in a theoretical model (Stevens and Zador, 1998). In these cases it has been found that dependence of synaptic inputs, not independence, generates variability and that synchronous synaptic inputs can be considered as candidates for the observed in vivo variability. The question arises of how synapse unreliability is compatible with the capability of neuronal networks, where signals have to cross different synapses, of processing information or performing behavioral tasks in a reproducible manner across different trials of the same input.

1.4 Aim of the work

When a touch stimulus is delivered to the skin of a leech segment many neurons in the ganglion directly innervating this segment and in the two neighboring ones fire action potentials (see Fig. 1.5) due to the organization of their receptive fields showing an extensive overlapping (see Fig. 1.3A).

In the first part of the thesis the simultaneous activation of mechanosensory neurons responsible for various sensory modalities was characterized. With a suitable experimental set-up (see chapter 2) it was possible to record in parallel the electrical activity of the majority of sensory neurons activated by touching the skin.

In particular the following questions were addressed:

i) how many mechanosensory neurons are activated by touching the skin in a given location?

ii) how reproducible and correlated is their electrical activity?

iii) how does the global firing pattern change with stimulus intensity and location?

iv) is this global firing pattern dynamical?

v) how does it change during adaptation?

The results are discussed in chapter 3.

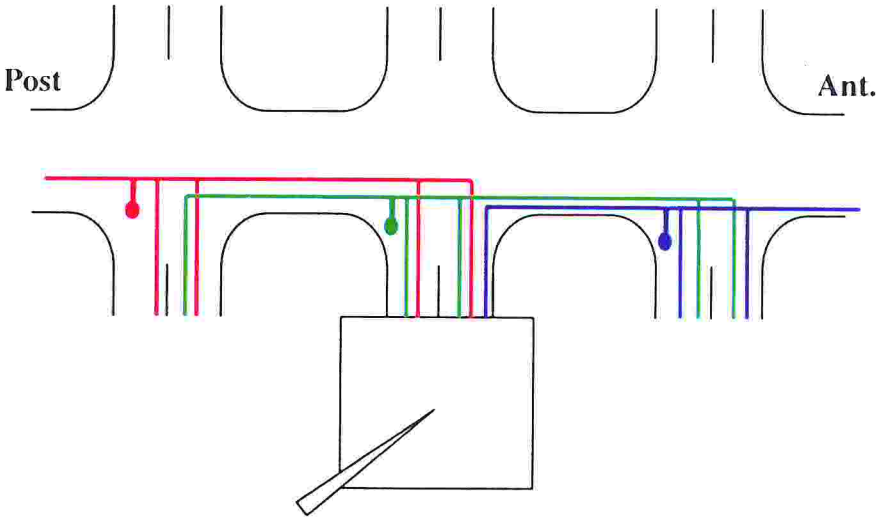

Fig. 1.5 Representation of a touch stimulus by the multiple activation of mechanosensory cells. Since the receptive fields of the same cells extend on three segments and receptive fields of different cells overlap, each point of the skin surface is redundantly innervated by several mechanosensory neurons.

In the second part of the thesis input-output relations in the isolated ganglion were studied exploiting the possibility to record multiple activation of neurons in parallel (see chapter 2). By evoking action potentials in identified mechanosensory cells there follows a clear response in motor neurons. The aim was to determine:

i) how many cells respond to the input?

ii) is it plausible to consider output as a distributed process?

iii) are responses of these cells reproducible from trial to trial?

iv) are responses correlated?

The answers to these questions allow to determine basic properties of the processing in the leech ganglion and shed light on how the information acquired by the sensory neurons can be transferred reliably to the output.

The results are discussed in chapter 4.

2 Materials and methods

Two sets of experiments were performed always in the leech CNS using different kinds of preparations and experimental protocols. In this chapter all the experimental procedures are explained with attention not only to technical questions but also to data analysis.

2.1 Experimental procedures

2.1.1 Animals and preparation

Specimens of *Hirudo medicinalis* were obtained from a commercial supplier (Ricarimpex, Eysines, France) and kept at 5° C in tap water dechlorinated by aeration for 24 hours. The leeches were dissected under a dissecting microscope. As mentioned above different kinds of preparation were arranged for various experiments.

For the study of sensory coding a semi-intact preparation was used, consisting of a chain of three segmental ganglia. The central ganglion of the chain innervated the skin of half a segment (either the left or the right one) (see Fig. 2.1) by the two roots (anterior and posterior). The roots (both dorsal and ventral branches) were carefully isolated from the connective tissue with sharp forceps. In this way the intact innervation of the skin was maintained, allowing to record en-passant through the application of suction electrodes.

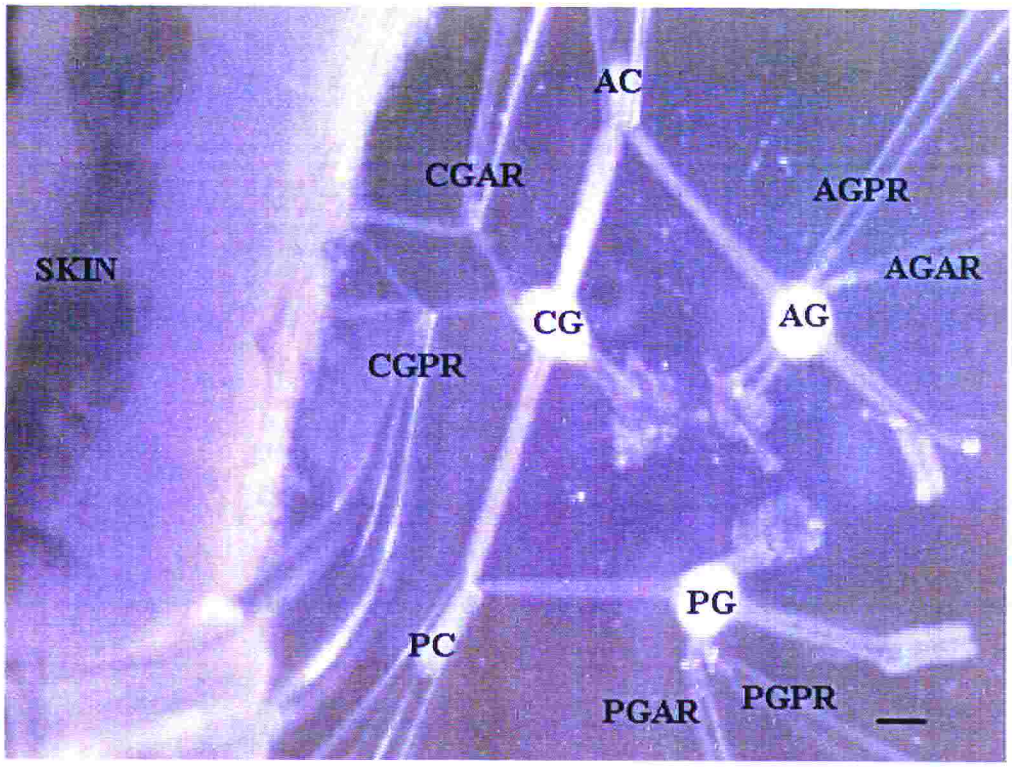

Figure 2.1 Semiintact preparation. A photograph of three leech ganglia with a piece of skin (SKIN) connected to the central ganglion (CG). 8 suction electrodes are used to obtain en-passant extracellular recordings from the anterior and posterior roots (CGAR and CGPR) and the two connectives (PC and AC) of the central ganglion and recordings from the posterior (PGPR and AGPR) and anterior roots (PGAR. and AGAR) of the anterior (AG) and posterior ganglion (PG). Scale bar (500 μm)

The preparation was then pinned in a Sylgard-coated dish to fix it mechanically and micromanipulators (Narishige) were used to apply the electrodes to the preparation.

For the experiments concerning the study of the input-output relations, a different kind of preparation was arranged (Fig. 2.2). In this case a single midbody ganglion (chosen between the 7^{th} and the 13^{th}) was isolated and connectives and roots (both ventral and dorsal) were cut in order to suck them into suction pipettes. The ganglion was pinned in a Sylgard coated dish with the ventral or dorsal side up.

Since mechanosensory neurons are contained in the ventral side of the ganglion, ventral side up configuration is suitable for impaling mechanosensory neurons. On the other hand since most motor neurons are contained in the dorsal side of the ganglion, dorsal side up configuration is useful when motor neurons have to be impaled.

In order to understand the terminology used to indicate the cells it has to be noticed that left (L) and right (R) neurons are defined with respect to an observer looking at the ganglion in ventral side up configuration with the anterior connective in the upper part of the visual field (as in Fig. 2.2).

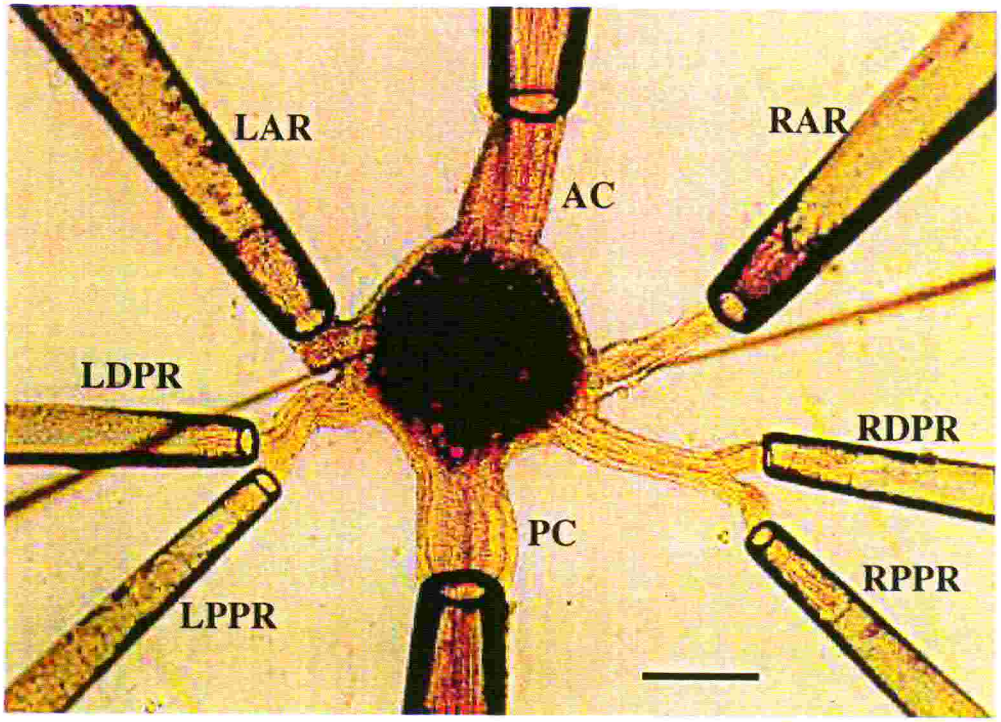

Figure 2.2 Isolated ganglion preparation. The left and right anterior (LAR, RAR), dorsal-posterior (LDPR, RDPR), posterior-posterior (LPPR, RPPR) roots bifurcating from the posterior roots (LPR, RPR) and the anterior and posterior connectives (AC, PC) of an isolated leech ganglion are drawn into suction pipettes. The sharper electrodes are intracellular electrodes just before commencing penetration in the ganglion. Scale bar 250 μm.

2.1.2 Solutions

During dissection, the preparation was bathed in a standard leech Ringer solution with the following composition (mM):

115.0 NaCl

1.8 $CaCl_2$

4.0 KCl

12.0 Glucose

10 Tris maleate buffered to pH 7.4 with NaOH.

During the experiment the preparation was perfused with the same standard Ringer solution.

For the experiment concerning the coding of mechanical stimulation it was necessary to isolate the contribution of mechanosensory neurons activated by the touch stimulus from that of other neurons involved in the response. In order to do this, the preparation was perfused with high Mg^{2+} Ringer solution. This solution was obtained by adding 15 mM $MgCl_2$ replacing 15 mM NaCl in the standard Ringer composition. The effect of bathing the preparation in this solution is the block of all chemical synapses.

All the experiments were done at room temperature (20-24°C).

2.1.3 Electrophysiological recordings

In all the experiments extracellular parallel recordings were performed in different parts of the preparation, so as to obtain recordings of action potentials from as many neurons as possible.

As shown in Fig. 2.1 and Fig. 2.2 different dispositions of extracellular suction pipettes were used in various experiments.

Coding of mechanical stimulation

In order to explore the response of all mechanosensory neurons activated by touching the skin of a single segment, a chain of three ganglia was used. The extracellular electrodes were placed so as to record action potentials from mechanosensory neurons activated in their major and minor receptive fields.

Eight glass suction pipettes were applied as shown in Fig. 2.1. Two pipettes of an inner diameter of 150 μm were applied en-passant onto the anterior and posterior connectives of the central ganglion, allowing to record action potentials invading branches of the neurons innervating the connectives. Another two suction pipettes of an inner diameter of 90 μm were used to record en-passant from the anterior and posterior roots innervating the skin. With these electrodes it was possible to observe action potentials originated in mechanosensory receptors located in the skin traveling towards the CNS. Finally, the last four suction pipettes, of an inner diameter of 80 μm, were applied onto the anterior and posterior roots of lateral ganglia in the chain, ipsilaterally to the piece of skin, allowing to clearly identify mechanosensory neurons activated by the stimulation of their minor receptive fields.

Input output relations

In the experiments aimed at characterizing variability of output responses in the ganglion I was interested in recording the activity of neurons evoked by the introduction of a sensory input in the ganglion.

Eight glass suction pipettes, six with an inner diameter of about 80.0 μm and two with an inner diameter of 120.0 μm, were used to suck the anterior roots, the bifurcations of posterior roots and the two connective fibers respectively (Fig. 2.2).

Two pipettes had to be used to record the activity in the posterior roots (applied to their bifurcations), since it became difficult to recognize the shapes of the signals produced by numerous action potentials occurring at the same time. In this way the action potentials were better resolved.

2.1.4 Intracellular recordings

The sensory input was mimicked by evoking action potentials in mechanosensory neurons injecting current with sharp intracellular electrodes.

The electrical activity of neurons was also monitored by intracellular recordings (Muller et al., 1981), obtained by impaling nerve cells with sharp electrodes. The electrodes were pulled using thin walled glass capillaries (WPI) and a P-97 (Sutter Instruments) puller. The input resistance range was 20-50 MΩ. Potassium acetate 4 M solution was used to fill the electrodes. The intracellular recordings were performed using an Axoclamp-2b amplifier (Axon Instruments, Foster City, CA).

2.1.5 Data collection and storage

Voltage recordings, either extracellular or intracellular, were digitized at 10 kHz and stored on a personal computer using pclamp8 program (Axon Instruments).

Voltage signals were also stored on an 8-channel digital audio recorder (DA-88 TASCAM).

2.1.6 Amplifiers

The design and realization of an 8-channel standard amplifier has been part of my PhD project. This amplifier is made up of two stages of amplification. The head-stage contains an 8-channel filter amplifier with a bandwidth of 200-2000 Hz and a gain of 10^3. The second stage of amplification contained in the rack mounted box has a gain ranging from 1 to 50. This amplifier was used to record the extracellular signals. The signal produced by the action potentials, recorded by the suction electrodes from the connectives and roots, ranged from 40 to 200 μV. The standard deviation of the noise was about 10 μV.

2.1.7 Optics

Impaling neurons under visual control was possible in dark field illumination, obtained by mounting a dark field condenser (Leitz Wetzlal) onto the microscope (Fixed stage upright, Olympus). A CCD camera was also mounted onto the microscope in order to collect images of the preparation when different neurons were impaled. In this way the identity of the neurons recorded could be checked in a second time.

2.1.8 Mechanical stimulation

Mechanical stimulation was delivered to the skin pressing nylon filaments driven by a solenoid (RS components), as described by Lewis and Kristan (1998a) following the Von Frey's method (Levin et al., 1978). Different stimulus intensities were obtained by changing the diameter and length of the filaments. A set of six nylon filaments delivering stimulus intensities of 1, 2.2, 6, 22, 50 and 100 mN was calibrated (see Table 1). The force exerted by each filament was measured with a force transducer (RS components). The solenoid driving the filaments was triggered with the recording start.

Force (mN)	Fil. Diameter (mm)	Fil. Length (mm)
1	0.16	90
2.2	0.16	30
6	0.2	230
22	0.2	160
50	0.2	90
100	0.4	180

Table 1: Strength of the touch stimulus and corresponding parameters of the filaments.

2.2 Data analysis

Since the problem addressed in my experiments was the parallel processing in the leech CNS, it was necessary to develop a suitable method for the analysis of the multi-electrodes recordings.

The possibility of discriminating the parallel excitation of different neurons is based on the hypothesis that action potentials produced by the same neuron have almost identical extracellular signals. Hence a crucial problem was the classification of the shapes of action potentials. In most of the experiments this task was accomplished in an automatic way. The development of a procedure to treat this problem automatically was part of my PhD work carried out in collaboration with other people in my laboratory who wrote the algorithms and the suitable routines. Routines for automatic spike sorting were realized in Matlab language.

2.2.1 Neuron sorting

The extracellular voltage signals (Fig. 2.3) were processed as follows: the noise of the recording was estimated by computing the standard deviation σ in the absence of any evident action potential (typically 10μV) and a threshold equal to 4σ was set (red line in Fig. 2.3 A). The events exceeding the threshold were selected (Fig. 2.3 B) and several quantities of these events were measured: the amplitudes h_1, h_2 and h_3 and the event duration are indicated in panel C. Three integrals I_1, I_2 and I_3 were also computed; I_i being the area of the wave corresponding to h_i.

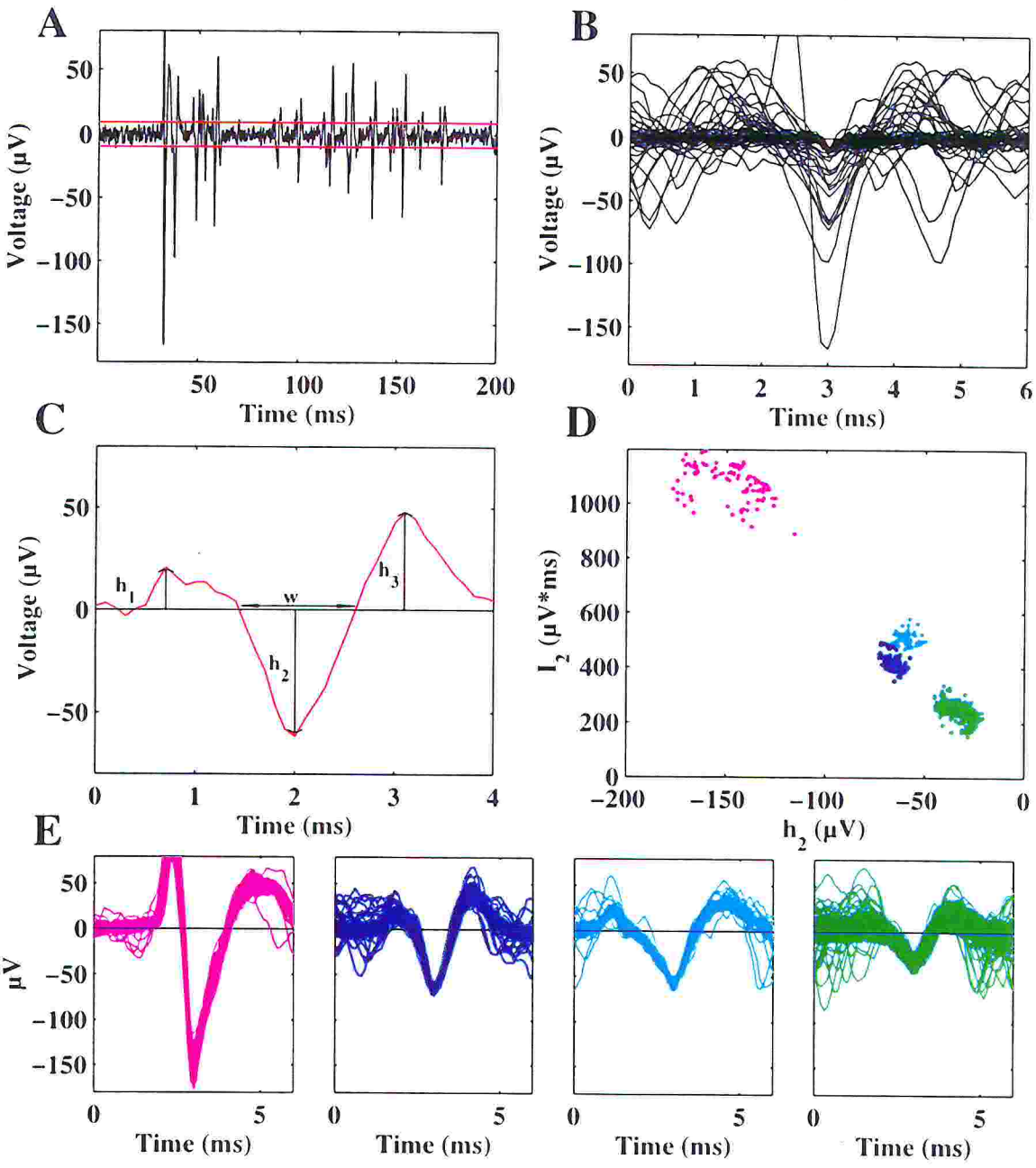

Fig. 2.3 Procedure for neuron sorting. A: An extracellular voltage recording obtained with a suction pipette. The red horizontal lines indicate the voltage level selected as threshold. B: Superimposed detected events. C: Quantities measured in a detected event. h_1, h_2 and h_3 are the amplitudes of the indicated waves; w is the event duration. Neurons were identified by grouping points in different plots. D: Plot of I_2 versus h_2. E: Neurons identified in an extracellular recording. Different colors indicate distinct neurons.

The neurons were identified by grouping events in different plots as shown in Fig. 2.3 D, where the plot I_2 versus h_2 was considered. Usually between 2 and 5 different neurons were reliably identified in each extracellular recording (Fig. 2.3 E).

A particular procedure was also developed to detect, automatically, the presence of signals in different channels with a fixed time delay which is a usual occurrence when neurons have their arborizations in more than one root or connective, so action potentials spread in the various branches of the axon and can be observed as exact delays from more than one suction pipette.

2.2.2 Motor neurons identification in the isolated ganglion

The automatic identification of action potentials was used in particular for the analysis of the experiments on the variability of evoked responses, since it was necessary to have a rich statistics of these responses.

After the application of the spike sorting procedure (see Fig. 2.3), the anatomical identification of the origin of sorted action potentials was obtained by the analysis of simultaneous intracellular and extracellular recordings of action potentials (see Fig. 2.4). The protocol of a typical experiment was the following: after the collection of many trials of the same stimulus the ventral side of the ganglion was first analyzed, the identified neurons (left and right AP, AE, Retzius cells, mechanosensory cells) were impaled under visual control and intracellular (spontaneous or evoked) action potentials were compared with signals observed with suction pipettes (see Fig. 2.4 A, first two columns).

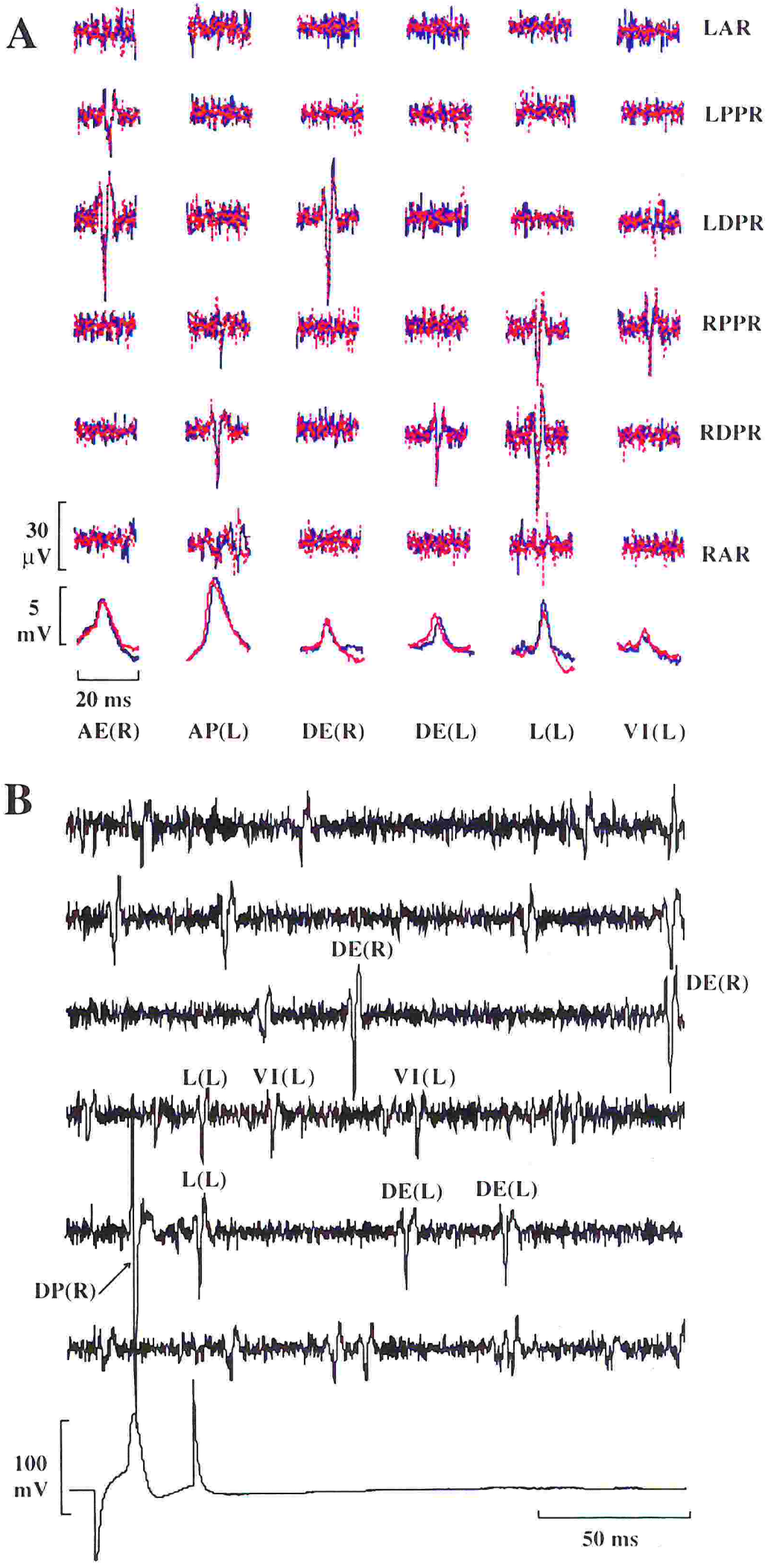
A
LAR
LPPR
LDPR
RPPR
RDPR
RAR
30 μV
5 mV
20 ms
AE(R)
AP(L)
DE(R)
DE(L)
L(L)
VI(L)
B
DE(R)
DE(R)
L(L)
VI(L)
VI(L)
L(L)
DE(L)
DE(L)
DP(R)
100 mV
50 ms

Fig. 2.4 Motor neuron identification. A: Extracellular recordings (from top to bottom) from the left anterior root (LAR), left posterior posterior root (LPPR), left dorsal posterior root (LDPR), right posterior posterior root (RPPR), right dorsal posterior root (RDPR) and right anterior root (RAR) and intracellular recordings (lower traces) when the neurons AE(R), AP(L), DE(R), DE(L), L(L) and VI(L) were impaled; two distinct recordings are shown (continuous blue plot and dashed red plot). B: Same extracellular recordings when the P_D(R) cell from the same ganglion was stimulated by inducing one action potential. Labels reported indicate action potentials of neurons, identified from the recordings shown in A.

Then the dorsal side of the ganglion was exposed for penetration and visually identified neurons were impaled. Finally, the intracellular and extracellular signals were analyzed and compared (see Fig. 2.4 A, last four columns).

T, P, and S cells produced typical large extracellular voltage signals and their action potentials were unambiguously identified from suction pipette recordings. Other neurons (such as the AP, L, N and AE cells) produced smaller extracellular signals, but on two, three and even four extracellular recordings, therefore their action potentials could be identified with good accuracy. When intracellular recordings from identified neurons and extracellular signals were simultaneously available (as in the experiment shown in Fig. 2.2) action potentials from the AP and the AE were identified from extracellular recordings with a precision of over 90% (as verified by comparison with intracellular recordings of action potentials). Large extracellular signals detected on the dorsal posterior root were identified as originating from DE and VI motor neurons after simultaneous intracellular and extracellular recordings. The exact shape and size of extracellular signals of an identified leech neuron varied in each experiment. However, identified leech neurons produced extracellular signals always on the same root.

2.2.3 Mechanosensory neurons identification in the semi-intact preparation

In the analysis of receptive fields the identification of mechanosensory neurons was done essentially by the visual inspection of the traces.

The electrical activity of each preparation (Fig. 2.1) was first checked in the usual extracellular solution (see above). If clear electrical signals (indicating the occurrence of action potentials) were recorded by all suction pipettes, the preparation was bathed in

high Mg^{2+} Ringer solution. After a few minutes the recorded electrical activity diminished and only the extracellular signals originating from mechanosensory neurons were recorded.

At the end of the experiments all mechanosensory neurons of the three ganglia ipsilateral to the skin were successively impaled with sharp intracellular electrodes. For each mechanosensory neuron action potentials were directly evoked by passing a depolarizing current pulse (see Fig. 2.5 B) and by touching the skin (Fig. 2.5 A). With this procedure it was possible to obtain a clear signature of extracellular voltage signals evoked by action potentials of each mechanosensory neuron.

These neurons have an extensive branching, as shown in Figs. 1.4 and 1.5 of the previous chapter, and the extracellular voltage signals associated with action potentials can be measured by suction pipettes placed at different locations, with an amplitude depending on the size of the axon at that location. Large extracellular signals were measured from the roots branching from the ganglion in which the recording was obtained (see Fig. 2.5 B). Clear and measurable extracellular voltage signals from the connectives were recorded only from T cells while those from P and N cells could be seen only by averaging different trials and were usually very small. Dorsal T and P cells (T_d and P_d) produced extracellular signals only in posterior roots, while other T and P cells (T_l, T_v and P_v) had signals in both roots. Action potentials of N cells (N_m and N_l) produced smaller voltage signals in both roots. These observations suggest some general rules for a preliminary identification of extracellular voltage signals: action potentials of T cells produce large signals in the roots and in one or two connectives (occasionally also in the roots of adjacent ganglia). Action potentials of P cells can be distinguished by a large signal in the roots not associated with a signal on the

connectives. Action potentials of N cells are usually smaller and they are less clearly identified.

The shapes of extracellular signals of action potentials evoked by touching the skin (Fig. 2.5 A) and by passing depolarizing current into the soma (Fig. 2.5 B) were almost identical, unlike the relative timing at which action potentials invade various branches of the neurons. In fact, extracellular signals of an action potential from a P_v cell in the central ganglion evoked by passing depolarizing current into the cell body were almost simultaneous on the anterior and posterior roots, but not when the action potential was evoked by touching the skin. In the case illustrated in Fig. 2.5 A (second column) the action potential is first detected in the anterior root, then in the cell body (see intracellular recording) and finally in the posterior root. This different timing reflects the propagation of the action potential from the endings of the anterior branches to the cell body and onto the posterior root. In some experiments it was possible to observe an inversion of the timing of signals on the two roots, when the skin near the midline was touched: sometimes the wave on the anterior root preceded that on the posterior one and vice-versa other times. This inversion is an indication of the variability of the locus of initiation of the action potential.

In the case of mechanosensory neurons belonging to adjacent ganglia (anterior or posterior) activated by the stimulation of their minor receptive fields (Fig. 2.5 A first and third column), the first action potential is detected in the connective (anterior or posterior) then in the cell body (see intracellular recordings) and finally in the roots of the anterior or posterior ganglion simultaneously.

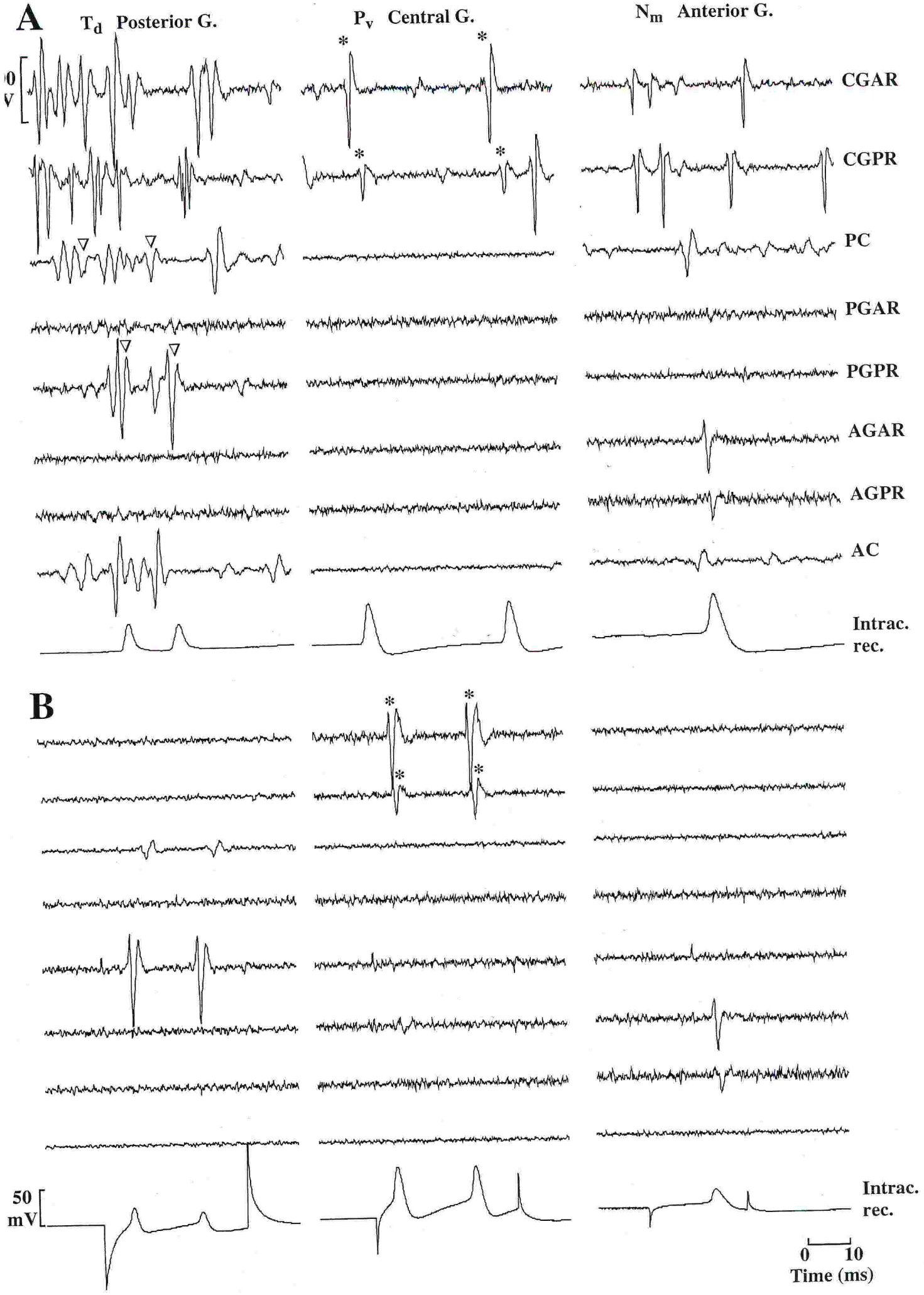
A
T_d Posterior G.
P_v Central G.
N_m Anterior G.
CGAR
CGPR
PC
PGAR
PGPR
AGAR
AGPR
AC
Intrac. rec.
B
50 mV
Intrac. rec.
0 10
Time (ms)

Fig. 2.5 Mechanosensory neurons identification. Recordings from 8 suction pipettes (1^{st} to 8^{th} traces) and one intracellular electrode (lowest trace): touching the skin (A) and passing current (B). Fig. 2 shows recordings from a T_d cell of the posterior ganglion (1^{st} column), from a P_v cell of the central ganglion (2^{nd} column) and from an N_m cell of the anterior ganglion (3^{rd} column). Labels for extracellular recordings were the same as those used in Fig. 2.1. Waveforms indicated by symbols are the extracellular voltage signals of action potentials of the impaled mechanosensory neurons.

Neuron identification was done in a semiautomatic way: different shapes of action potentials were classified as described in the previous section and pairs or triplets of extracellular action potentials were detected. In some cases action potentials of two different mechanosensory neurons occurred simultaneously, so that their extracellular signals had an unusual shape and size. In these cases, however, by knowing which location of the skin was stimulated with which intensity, it was often possible to identify mechanosensory neurons (see for instance upper trace of Fig. 3.5C). When different T and P cells were simultaneously active a reliable neuron identification was not possible from extracellular recordings. In these cases the statistics of each neuron was recovered from intracellular recordings from visually identified mechanosensory neurons (see for instance Fig. 3.2 or Fig. 3.10). The final validation of the identity of the neuron was made by visual inspection.

3 Coding of mechanical stimulation

The first stage of information processing taking place in the nervous system is represented by the coding of a sensory input in the electrical activity of primary sensory neurons.

The leech CNS allows an easier approach to the problem of mechanosensory coding from the experimental point of view. Indeed, this system, as discussed in chapter 1, is simply organized, in particular mechanosensory neurons are recognizable for their position and anatomy, their electrical activity is easy to monitor by intracellular and extracellular electrodes and their physiology has been characterized in details as reviewed in chapter 1. This chapter reports the results obtained from experiments aimed at characterizing simultaneous activation of the mechanosensory neurons in a semi-intact preparation consisting of three adjacent leech ganglia innervating a single segment of the skin, as illustrated in Fig. 2.1. Touching the skin in a given location usually causes the activation of several mechanosensory neurons, both in the ganglion directly innervating skin of the segment touched (central ganglion) and in the adjacent ones. As already discussed in chapter 1, mechanosensory neurons innervate not only the skin segment corresponding to the ganglion in which their cell bodies are contained (major receptive fields), but also the segments belonging to the adjacent ganglia (minor receptive fields) by branches that run through the anterior and posterior connective and then through the roots of adjacent ganglia (Yau, 1976).

Therefore each point of the skin belongs to at least three distinct receptive fields of the corresponding cells. Moreover, since each ganglion contains more than one mechanosensory neuron and their receptive fields overlap (Nicholls and Baylor, 1968; Blackshaw, 1981), the representation or coding of the simplest touch stimulus involves usually from 5 to 15 cells. In this respect, I studied how a sensory input is coded by a population of sensory neurons. More precisely, I tried to understand the extent and the characterization of the multiple activation of these cells (such as their reproducibility, latency, jitters and adaptation), whether mechanosensory coding can be considered as a distributed process and whether any strategy for neural processing of the input can be retrieved from this architecture.

3.1 Results

As illustrated in Fig. 2.1, a preparation was obtained and a suitable experimental set-up allowed to record the simultaneous activation of mechanosensory neurons in response to a touch stimulus of the skin. The action potentials in the mechanosensory neurons were recorded with eight extracellular suction pipettes and one or two intracellular electrodes (see chapter 2). The stimulation of the skin was obtained by pressing nylon filaments fixed to a rod driven by a solenoid. The touch stimulus delivered was set by changing the duration, the location and the intensity of the force exerted. In this way it was possible to check the selective activation of different kinds of mechanosensory neurons, to determine their receptive fields and the dynamics of their activation.

When the skin was touched for about 80 ms with a filament exerting a force of 1 mN, one or more T cells belonging to the central ganglion fired one or two action potentials. The T cells from adjacent ganglia also fired action potentials. The timing of the first evoked action potential of a T cell was measured and it was very reproducible with a jitter of less than 1 ms. When the force exerted by the filament increased above 20 mN, P cells of the central and adjacent ganglia fired action potentials. The action potentials in P cells usually followed those evoked in T cells with a delay of about 20 ms and had a larger jitter of about 5 ms. Stronger stimuli exceeding 50 mN caused also the N cells firing. In such a régime of stimulation the majority of mechanosensory neurons of the three ganglia fired action potentials. The spatial properties of the receptive fields of mechanosensory neurons were explored by touching different parts of the skin. When the mechanical stimulation was applied for a longer time, i.e. 1 s, the activation of mechanosensory neurons was observed. T cells in all ganglia fired action potentials just at the transient of the stimulus (Carlton and Mc Vean, 1995). P cells responded in a different way if activated in their major or minor receptive fields. P cells of the central ganglion continued to fire action potentials during the entire stimulation, while P cells of adjacent ganglia quickly adapted after about 100 ms of steady mechanical stimulation. Therefore for normal prolonged stimulation, at the steady state, only P cells of the central ganglion fired action potentials. N cells were also activated at the steady state for higher stimulus intensities. One of the hypotheses that can be done to justify adaptation of P cells in adjacent ganglia is that of conduction block (Van Essen, 1973; Yau, 1974; Macagno et al., 1987; Gu, 1991). This hypothesis can be rejected since P cells of adjacent ganglia can still fire action potentials during the period of adaptation if a mechanical stimulation is introduced in a second location on the skin.

These results indicate basic properties of coding of mechanical stimulation in leech mechanosensory neurons: a brief and localized stimulation of the skin involves the activation of more than a dozen different mechanosensory neurons in three adjacent ganglia; after 100 ms of steady stimulation most of these cells adapt. Adaptation occurs primarily at the nerve endings and mechanosensory neurons can quickly respond when a second mechanical stimulation is introduced in a different location.

3.1.1 Parallel recordings from mechanosensory neurons

In order clearly to resolve action potentials coming from mechanosensory neurons in the extracellular recordings the preparation was perfused with a Ringer solution containing 15 mM $MgCl_2$ (Nicholls and Baylor, 1968). In this medium chemical synapses were blocked and the mechanical stimulation of the skin activated only sensory neurons, so it was possible reliably to identify extracellular action potentials produced by identified mechanosensory neurons.

Fig. 3.1 shows two sets of eight extracellular recordings obtained in the absence (A) and in the presence (B) of Mg^{2+} in the Ringer solution.

Before the perfusion with Mg^{2+} (Fig. 3.1A) the action potentials evoked in mechanosensory neurons by touching the skin are superimposed to evoked responses of other neurons (including motor neurons) and spontaneous electrical activity mediated by chemical synapses. Moreover, since the preparation includes muscles, their contraction usually produces a feedback on mechanosensory neurons, leading to an additional response in these cells.

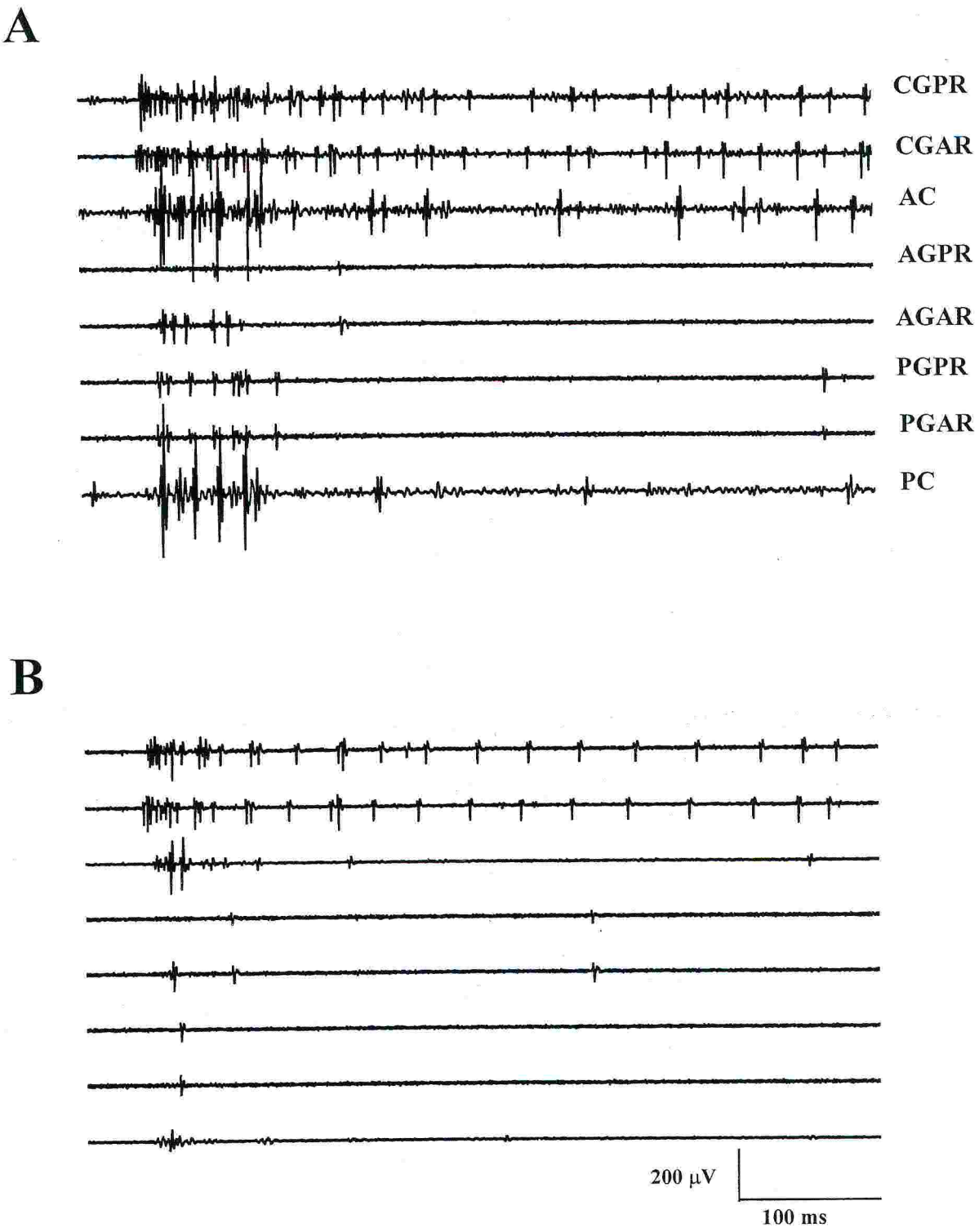

Fig. 3.1 Comparison of responses to touch stimuli in the absence and in the presence of Mg^{2+} in the extracellular medium. A: eight extracellular recordings obtained in the experiment illustrated in Fig. 2.1 bathing the preparation in normal Ringer fluid; labels refer to the extracellular electrodes illustrated in Fig. 2.1. B: eight extracellular recordings as in panel A, when the preparation was bathed in high Mg^{2+} Ringer fluid.

After a few minutes of perfusion with high Mg^{2+} Ringer solution (Fig. 3.1 B), the same stimulation of the skin evoked responses just in mechanosensory neurons.

Fig. 3.2 illustrates a set of extracellular recordings obtained with eight suction pipettes when the skin was touched with a filament exerting a force equivalent to 50 mN for 80 ms. The recordings were carefully examined following the procedure explained in section 2.2.3. The action potentials of the identified mechanosensory neurons have been indicated with appropriate symbols (see box in Fig. 3.2) and colors (blue, red and green for mechanosensory neurons in the central, posterior and anterior ganglion respectively). Both T and P cells are activated by this mechanical stimulation. The two lowest traces are five superimposed intracellular recordings from the T_v cell and P_v cell of the central ganglion.

From the intracellular and extracellular traces it appears evident that the first action potential of the ventral T_v cell occurs with a jitter of 0.8 ms, while the first action potential of the dorsal P_d cell has a larger jitter of 4.5 ms.

As expected from the anatomy of nerve endings (Blackshaw, 1981), the most superficial endings of T cells are evidently the first to be stimulated and action potentials in these cells usually precede those from P and N cells with nerve endings located deeper in the skin (Blackshaw et al., 1982).

In each preparation studied in this experiment at least 10 different areas of the skin were stimulated and electrical responses were analyzed. In 76% of the recordings the first action potential evoked in T cells had a small jitter varying from 0.6 to 3 ms, while that of P cells had a slightly larger jitter ranging from 2.3 to 10 ms. Action potentials from N cells were detected with a stronger stimulation above 50 mN and the first action potential had a jitter varying between 5 and 15 ms (see section 3.1.3).

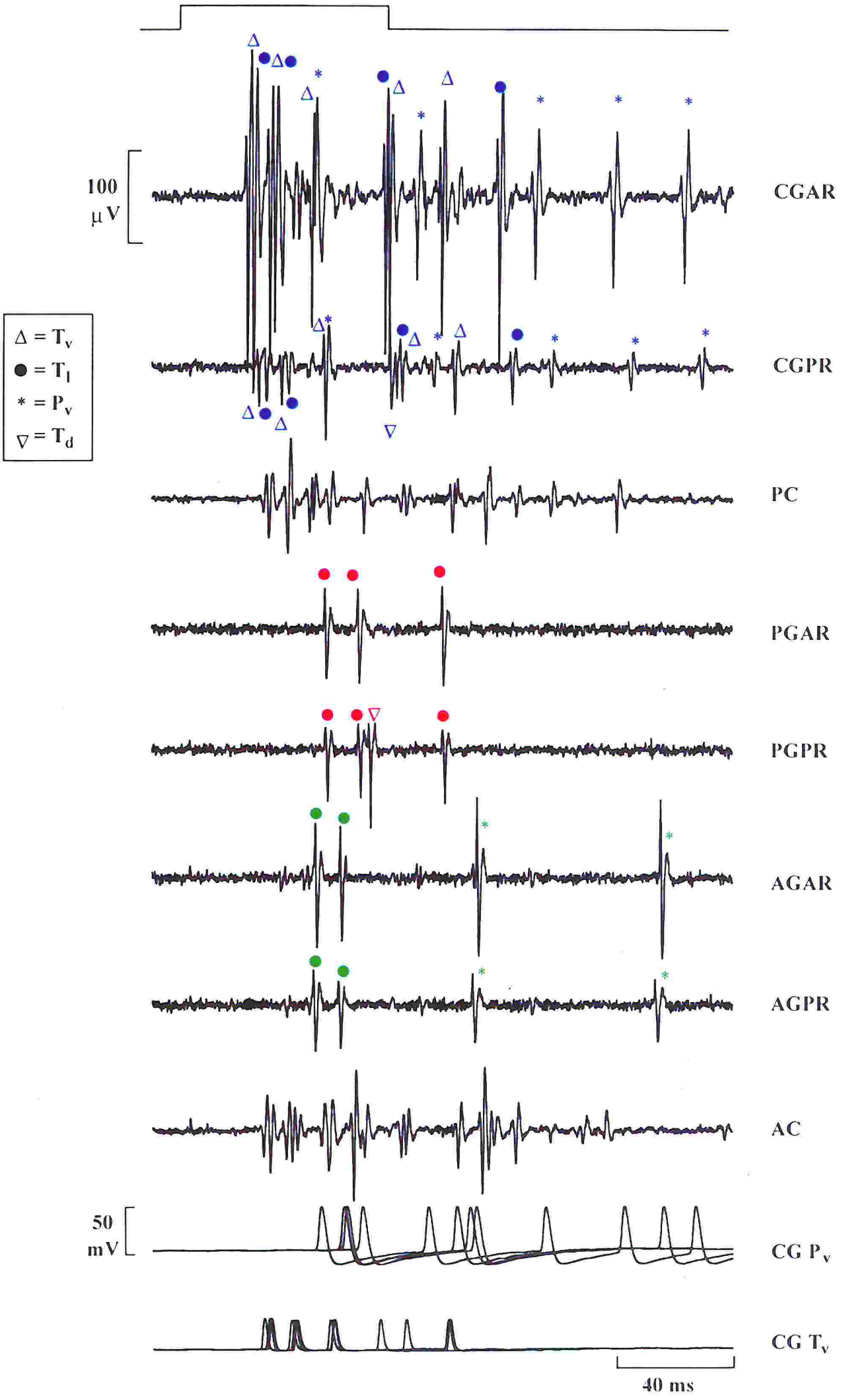
Δ = T_v
● = T_l
* = P_v
▽ = T_d
100 μV
CGAR
CGPR
PC
PGAR
PGPR
AGAR
AGPR
AC
50 mV
CG P_v
CG T_v
40 ms

Fig. 3.2 Eight extracellular and two simultaneous intracellular recordings from a P_v and a T_v cell (from top to bottom). 5 superimposed traces from the two intracellular electrodes. Stimulation of the skin with a filament exerting a force of 22 mN for 80 ms (as indicated by the top bar). Labels beside the traces are indicated as in Fig. 2.1. Action potentials from identified mechanosensory neurons are indicated with appropriate symbols (see box on the left) and colors (blue, red and green for mechanosensory neurons in the central, posterior and anterior ganglion respectively).

3.1.2 Receptive field properties with brief mechanical stimulation

The receptive field properties of different mechanosensory neurons were analyzed for brief (50 or 80 ms) stimulations of the skin. The piece of skin attached to the central ganglion was mapped in 12 or 20 distinct areas. This piece corresponded to the right or left hemisegment and was mapped into four regions from dorsal to ventral (D, DL, VL, V) and into five regions from anterior to posterior (A, P), corresponding to the five annuli of the body segment. Each distinct area of skin was touched with the filament at least 5 times and action potentials originating from identified mechanosensory neurons were counted and averaged in a time window of 100 ms following the stimulus onset. The receptive field was also mapped on a coarser grid with only three different regions from anterior to posterior. By stimulating 12 or 20 distinct areas, the same receptive fields were obtained, and therefore only 12 areas were tested in the majority of the experiments.

Fig. 3.3 illustrates four sets of extracellular recordings obtained in different areas of the skin for a brief mechanical stimulation (80 ms). Action potentials from identified mechanosensory neurons are indicated by appropriate symbols. These receptive fields, obtained with a stimulation exerting a force of 100 mN, are shown in Fig. 3.4. Panels A, B and C show the receptive fields for T, P and N cells of the central, posterior and anterior ganglion respectively. In all experiments aimed at mapping the receptive field, the neurons from the adjacent ganglia contributed.

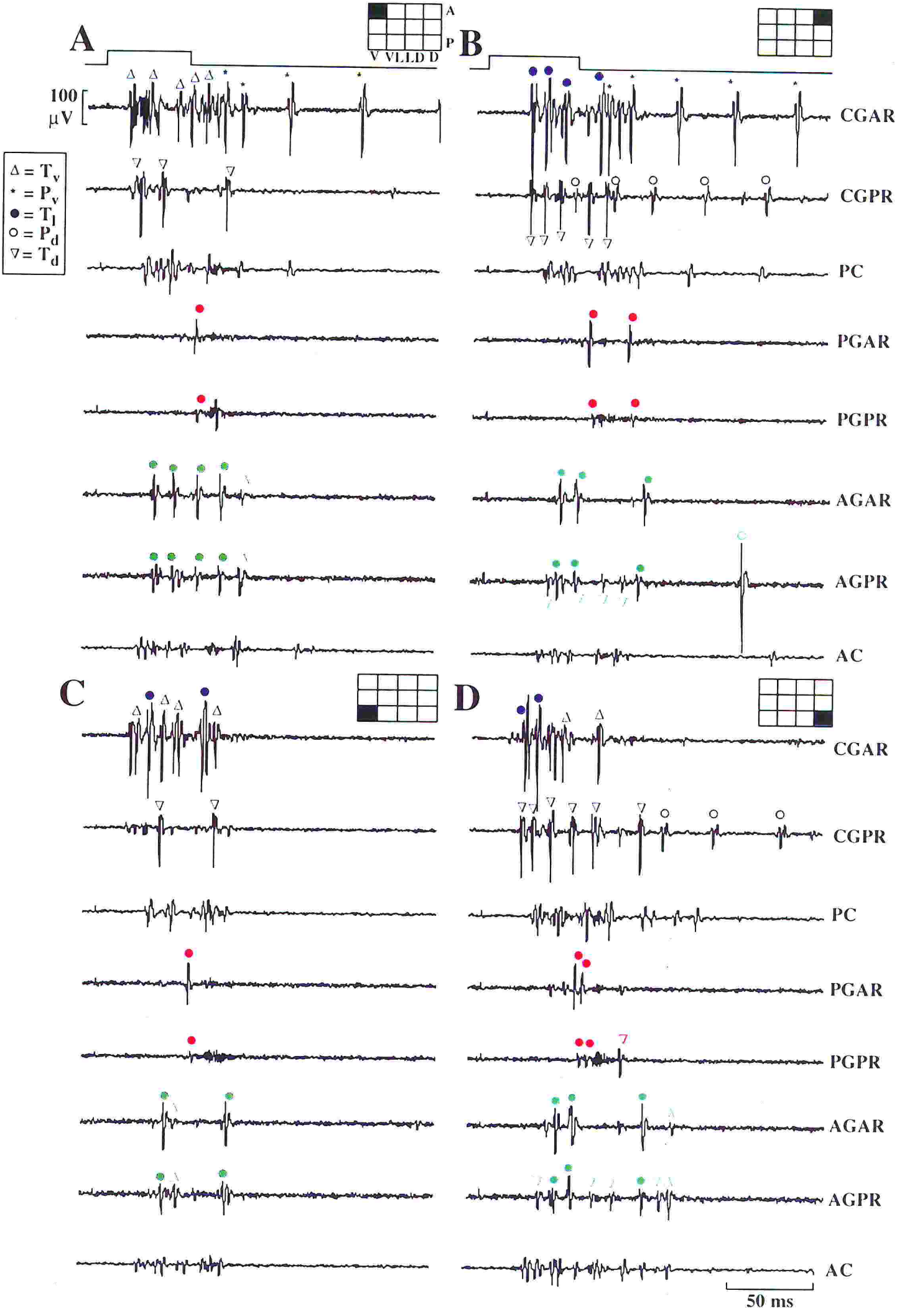
A
B
C
D
A
P
V VL LD D
100 µV
Δ = T_v
⋆ = P_v
● = T_l
○ = P_d
▽ = T_d
CGAR
CGPR
PC
PGAR
PGPR
AGAR
AGPR
AC
50 ms

Fig. 3.3 Coding of stimulus location. A, B, C and D: recordings from suction pipettes at four different areas of the half body segment, as indicated in the inset (V = ventral, VL = ventro-lateral, LD = latero-dorsal, D = dorsal, A = anterior, P = posterior). Stimulus intensity was 20 mN with a duration of 80 ms (as indicated by the top bar). Labels beside the traces as in Fig. 2.1. Action potentials from identified mechanosensory neurons are labeled as in Fig. 3.2.

During the initial 100 ms the number of action potentials of the T cells from the anterior and posterior ganglion was 23±5 % and 32±7 % of those recorded from the central ganglion. For P cells these values were 16±5 % and 18±7 % respectively. As a consequence the ratio between the number of action potentials from mechanosensory neurons in adjacent ganglia and in the central ganglion was 0.55 and 0.33 for T and P cells respectively.

The responses of neurons from adjacent ganglia were usually less pronounced, with a less distinction between ventral and dorsal. This spatial profile was not as smooth as that of neurons from the central ganglion and it was often possible to find a region with a very low sensitivity, surrounded by regions with an appreciable sensitivity. While the receptive fields of mechanosensory neurons from the central ganglion were remarkably reproducible in different experiments, those of neurons from adjacent ganglia were variable.

As already known (Nicholls and Baylor, 1968) when the ventral side of the skin is touched (see Fig. 3.3 A and C) many action potentials of T_v, P_v and T_l cells are detected, but also action potentials of the T_d cell of the central ganglion appear. Similarly, when the dorsal side of the skin is touched (see Fig. 3.3 B and D) action potentials from T_d,T_l and P_d cells are primarily observed, but occasionally also those from T_v and P_v cells appear.

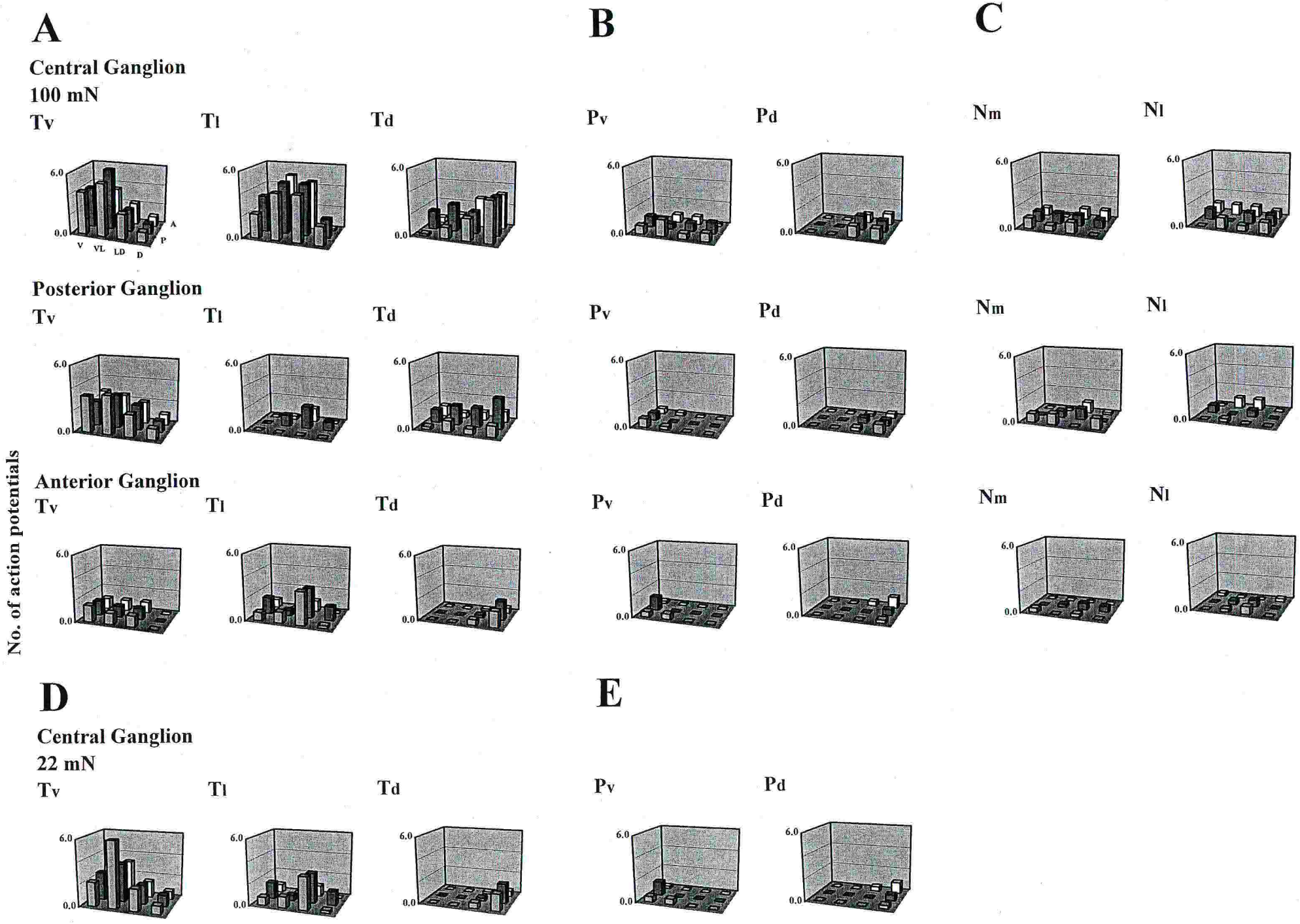
A
Central Ganglion
100 mN
Tv
Tl
Td
6.0
0.0
V
VL
LD
D
A
P
Posterior Ganglion
Tv
Tl
Td
Anterior Ganglion
Tv
Tl
Td
No. of action potentials
B
Pv
Pd
Pv
Pd
Pv
Pd
C
Nm
Nl
Nm
Nl
Nm
Nl
D
Central Ganglion
22 mN
Tv
Tl
Td
E
Pv
Pd

Fig. 3.4 Receptive field for a brief stimulation of different mechanosensory neurons. T (A), P (B) and N (C) cells of the central, posterior and anterior ganglion for a stimulation corresponding to 100 mN. Each graph reproduces the receptive field for a given mechanosensory neuron. D, E: Receptive fields of T(D) and P (E) cells for a stimulation corresponding to 22 mN. The receptive field was obtained by computing the average number of action potentials in a time window of 100 ms after the onset of the stimulation. Each stimulation was repeated at least 8 times.

Panels D and E of Fig. 3.4 reproduce respectively the receptive field of the three T cells and the two P cells of the central ganglion obtained with a lighter stimulation corresponding to 22 mN. The receptive fields obtained with the two stimulations of 22 and 100 mN are very similar. Therefore the spatial profile of the receptive field does not depend much on the intensity of the stimulus used to map it.

It is necessary to specify that the receptive fields of the cells here described are not the real receptive fields; indeed the analysis was limited to a single segment of skin, while each mechanosensory neuron innervates three adjacent segments (Nicholls and Baylor, 1968; Yau, 1976). Therefore the results here reported (Figs. 3.3 and 3.4) characterize the major receptive fields for the neurons in the central ganglion and the minor receptive fields for those in the adjacent ones.

3.1.3 Dependence of sensory responses on stimulus strength

When the skin was touched with filaments exerting forces of increasing strength different mechanosensory neurons were activated. As shown in Fig. 3.5 A, when the skin was lightly touched with a filament exerting a force of 1 mN on the ventral side, two action potentials of the T_v cell and one action potential of the T_l cell of the central ganglion were observed, but also an action potential from a T_v cell of the anterior ganglion appeared.

Increasing the stimulus intensity to 2.2 mN evoked a larger number of action potentials from T cells of the central ganglion (see Fig. 3.5B) and with a stimulus intensity of 6 mN the T_v cell of the posterior ganglion was also activated (see Fig. 3.5C). When the stimulus intensity was increased to 22 mN, P_v cells of the three ganglia started to fire action potentials and T cells fired more vigorously (see Fig. 3.5D).

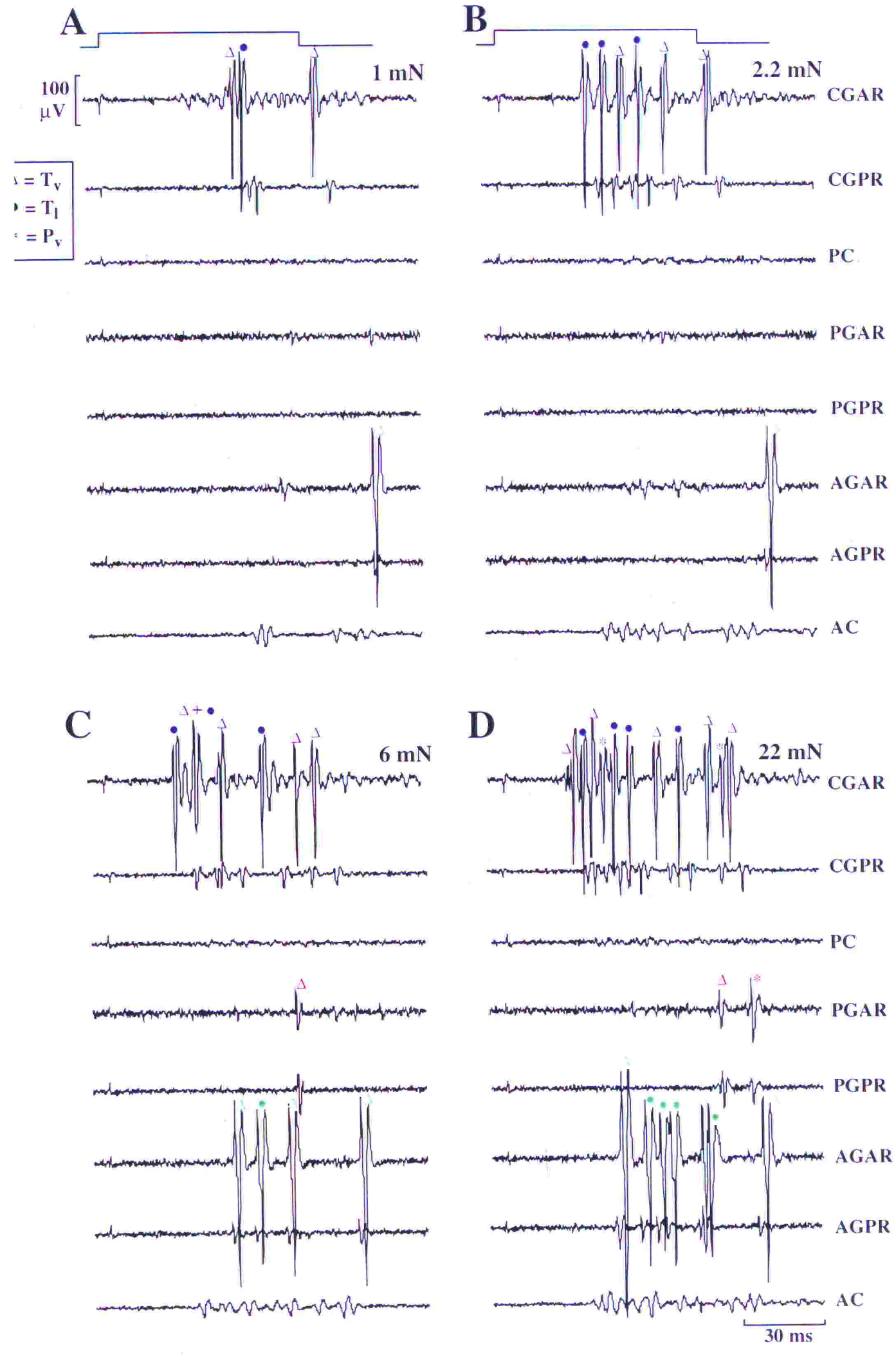
A
1 mN
100 µV
= T_v
= T_l
= P_v
B
2.2 mN
CGAR
CGPR
PC
PGAR
PGPR
AGAR
AGPR
AC
C
6 mN
D
22 mN
CGAR
CGPR
PC
PGAR
PGPR
AGAR
AGPR
AC
30 ms

Fig. 3.5 Responses to stimuli of increasing strength. A, B, C and D: recordings from suction pipettes with stimulus exerting 1, 2.2, 6 and 22 mN respectively. Stimulus duration was 80 ms (as indicated by the top bar). Labels beside the traces as in Fig. 2.1. Action potentials from identified mechanosensory neurons are labeled as in Fig. 3.2.

The dependence of the evoked activity on the stimulus intensity is analyzed in more detail in Fig. 3.6. Panel A reproduces the average number of action potentials, as a function of stimulus intensity, in a time window of 100 ms following the stimulus onset for mechanosensory neurons of the central (top), posterior (middle) and anterior ganglion (bottom). Panels B and C reproduce the latency and the jitter of the first evoked action potential, respectively. The latency of the action potentials evoked by the mechanical stimulation was measured as the delay between the onset of the mechanical stimulation and the peak of the action potential, measured either intracellularly or extracellularly. The onset of the mechanical stimulation is taken as the onset of the voltage pulse used to trigger the solenoid driving the filament motion. As the filament touches the skin some ms after the application of the voltage pulse to the solenoid, the latency as defined above is some ms longer than the interval between the time at which the skin is first touched and the time of the action potential occurrence. The jitter of the first evoked action potential is the standard deviation of the latency as defined above.

The threshold for activation of mechanosensory neurons in the central ganglion is very similar to that already reported (Nicholls and Baylor, 1968; Lewis and Kristan, 1998b) and is below 2 mN for T cells, about 20 mN for P cells and about 50 mN for N cells (Nicholls and Baylor, 1968). As shown in Figs. 3.5 and 3.6 the threshold for the activation of mechanosensory neurons of adjacent ganglia is slightly higher than that for neurons of the central ganglion.

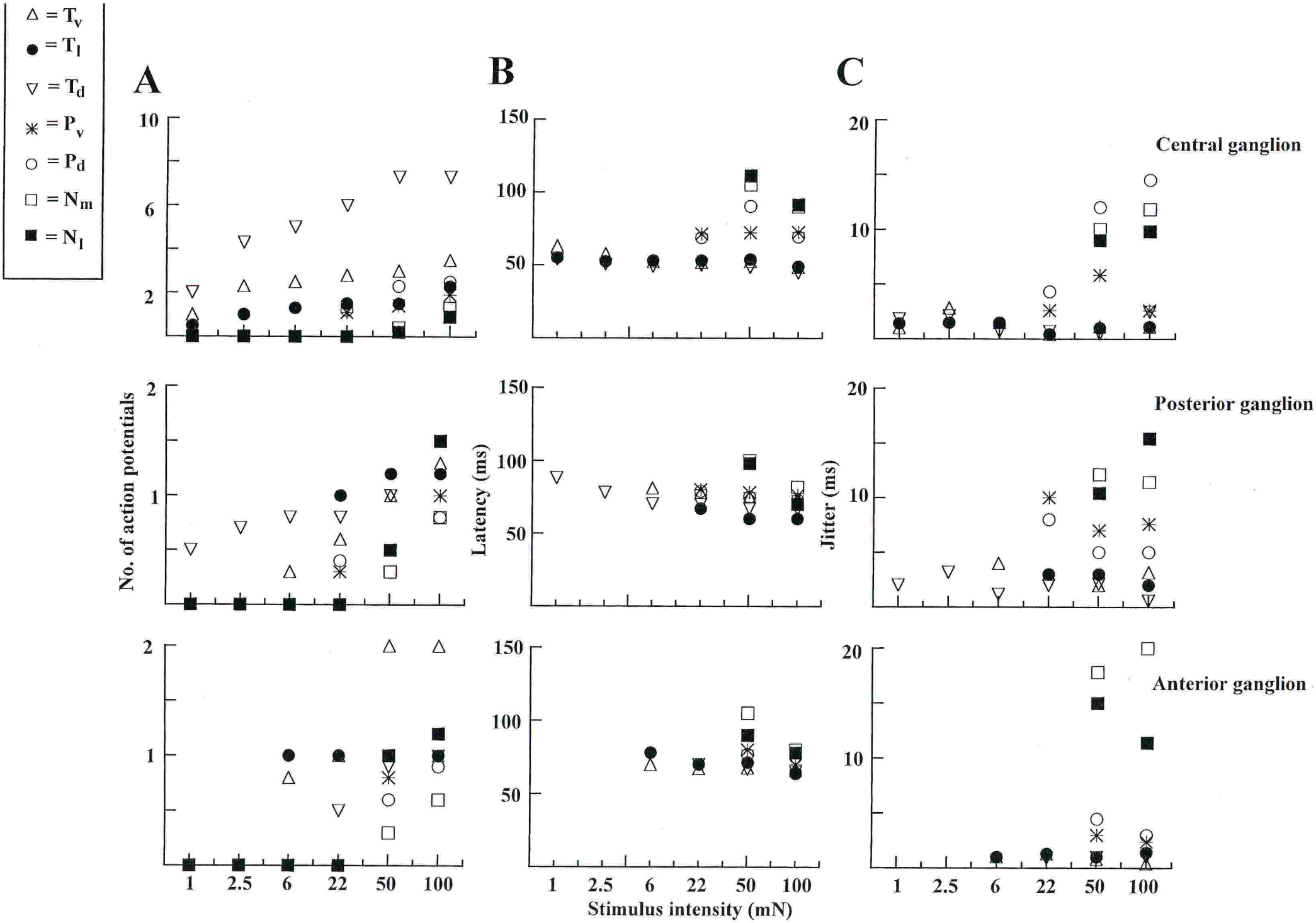
△ = T_v
● = T_l
▽ = T_d
✳ = P_v
○ = P_d
□ = N_m
■ = N_l
A
B
C
Central ganglion
Posterior ganglion
Anterior ganglion
No. of action potentials
Latency (ms)
Jitter (ms)
Stimulus intensity (mN)
1
2.5
6
22
50
100

Fig. 3.6 Number of action potentials, latency and jitter as a function of stimulus strength for mechanosensory neurons. A: number of evoked action potentials - in a time window of 100 ms after the onset of the stimulation - as a function of stimulus intensity in the central, posterior and anterior ganglion. B: latency of the first evoked action potential as a function of stimulus intensity in the central, posterior and anterior ganglion. C: jitter (i.e. standard deviation of latency) of the first evoked action potential as a function of stimulus intensity in the central, posterior and anterior ganglion. The statistics for N cells was based on intracellular recordings and not on extracellular signals, as they were too small to be detected during periods of intense electrical activity.

The latency of the first evoked action potential of T cells decreased from about 55 ms to about 40 ms when the stimulus intensity was increased from 1 to about 20 mN (see Figs. 3.5 and 3.6), but the decline was less clear at higher intensities above 50 mN. The first action potential evoked in P cells usually followed by about 20 ms the first action potential evoked in T cells and its latency did not change appreciably when the stimulus intensity was increased from 22 to 100 mN.

The jitter of all mechanosensory neurons did not depend significantly on the stimulus intensity and was characteristic for each type of neuron. The jitter of the first evoked action potential was on average 1.8 ms (ranging from 0.6 to 3 ms) for T cells and 3.5 ms (ranging from 2.3 to 10 ms) for P cells. N cells had an average jitter of 11.5 ms, varying between 8 and 18 ms (see Fig. 3.6C).

3.1.4 Responses to prolonged stimulation

The receptive field properties of different mechanosensory neurons were analyzed for prolonged stimulation of the skin (1 s). In this case the receptive field was characterized by computing the average firing frequency during the steady state of the stimulus.

In these case the recordings shown in Fig. 3.7 were obtained.

Panels A and C show extracellular recordings obtained when the mechanical stimulation was applied to a ventral area of the skin, indicated in the panel inset, while B and D represent responses to dorsal stimulation. Panels A and B show recordings in response to a 22 mN stimulus, while C and D are to a 100 mN stimulus.

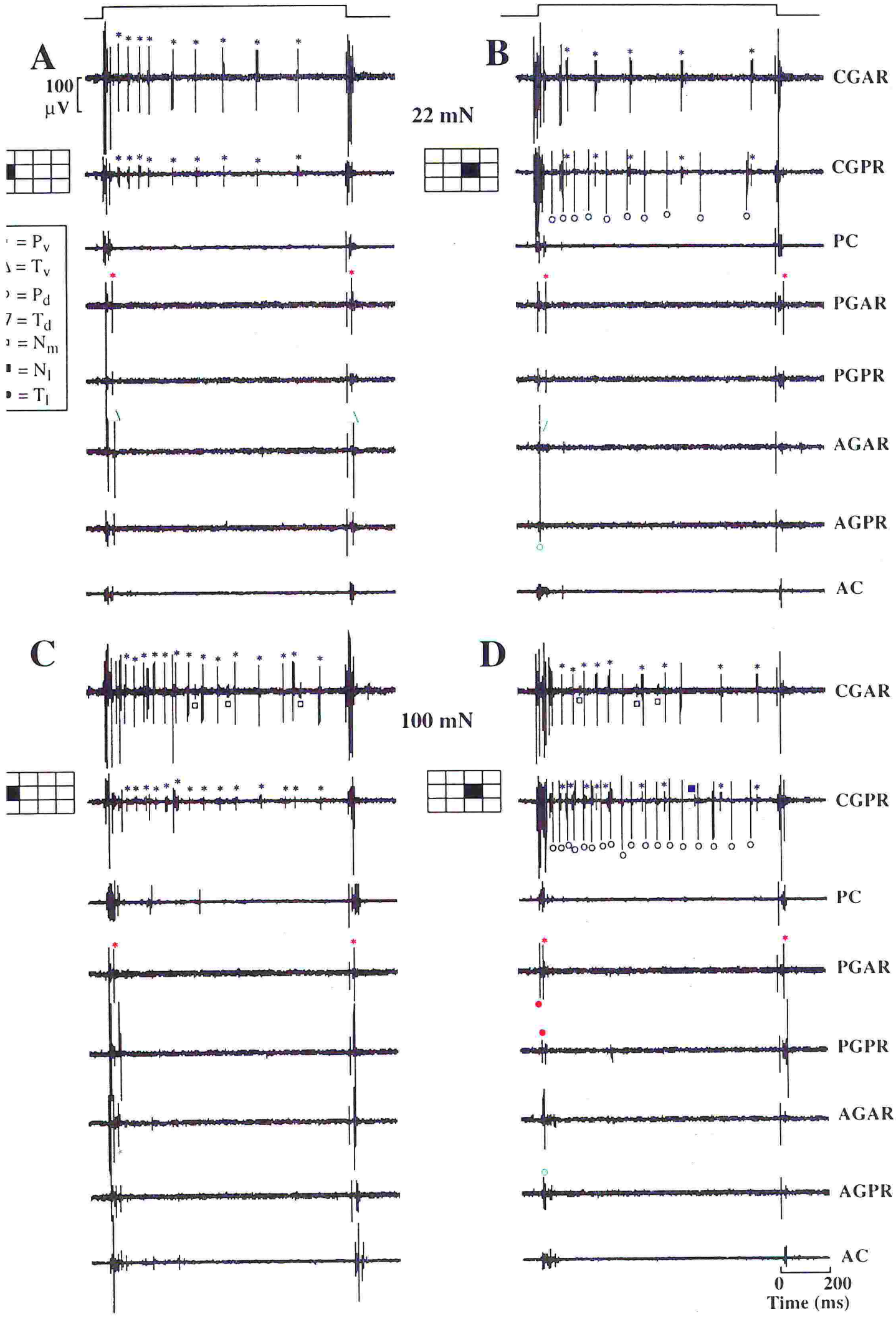
A
100
μV
= P_v
= T_v
= P_d
= T_d
= N_m
= N_l
= T_l
22 mN
B
CGAR
CGPR
PC
PGAR
PGPR
AGAR
AGPR
AC
C
100 mN
D
CGAR
CGPR
PC
PGAR
PGPR
AGAR
AGPR
AC
0
200
Time (ms)

Fig. 3.7 Responses to stimulation in two different areas and at two stimulus intensities. The two locations where the stimulus was applied are indicated in the panel inset and the stimulus intensity was 22 and 100 mN in A, B and C, D respectively. Stimulus duration was 1 s (as indicated by the top bar). Labels beside the traces as in in Fig. 2.1. Action potentials from identified mechanosensory neurons are labeled as in Fig. 3.2.

With a prolonged mechanical stimulation, T and P cells of the anterior and posterior ganglia quickly adapted, while P cells of the central ganglion did not. In the presence of a stimulation corresponding to 22 mN, P cells of the central ganglion fired at about 10 Hz, while stronger stimulation of 100 mN increased the rate to about 16 Hz. T, P and N cells (see also Fig. 3.10D, E and F) responded well and reproducibly to the stimulus cessation at both stimulus intensities.

Fig. 3.8 shows receptive fields for P and N cells during adaptation. Panels A and B reproduce data obtained for P cells and N cells respectively, for the central, posterior and anterior ganglion during a stimulation of 100 mN. Panel C illustrates receptive fields for P cells for the central ganglion during a stimulation of 22 mN, not activating N cells. These receptive fields are very similar to those obtained with a brief stimulation (see Fig. 3.4) with the exception of T cells (not shown) and P cells of adjacent ganglia (null histograms) which do not fire action potentials in the steady state.

The different adaptation of P cells of the central ganglion and of adjacent ganglia was observed in 11 preparations, which were tested in 12 different regions of the skin. In one preparation, however, a prolonged stimulation of the skin on two specific regions produced a sustained discharge of 3 to 5 Hz also in P cells of adjacent ganglia.

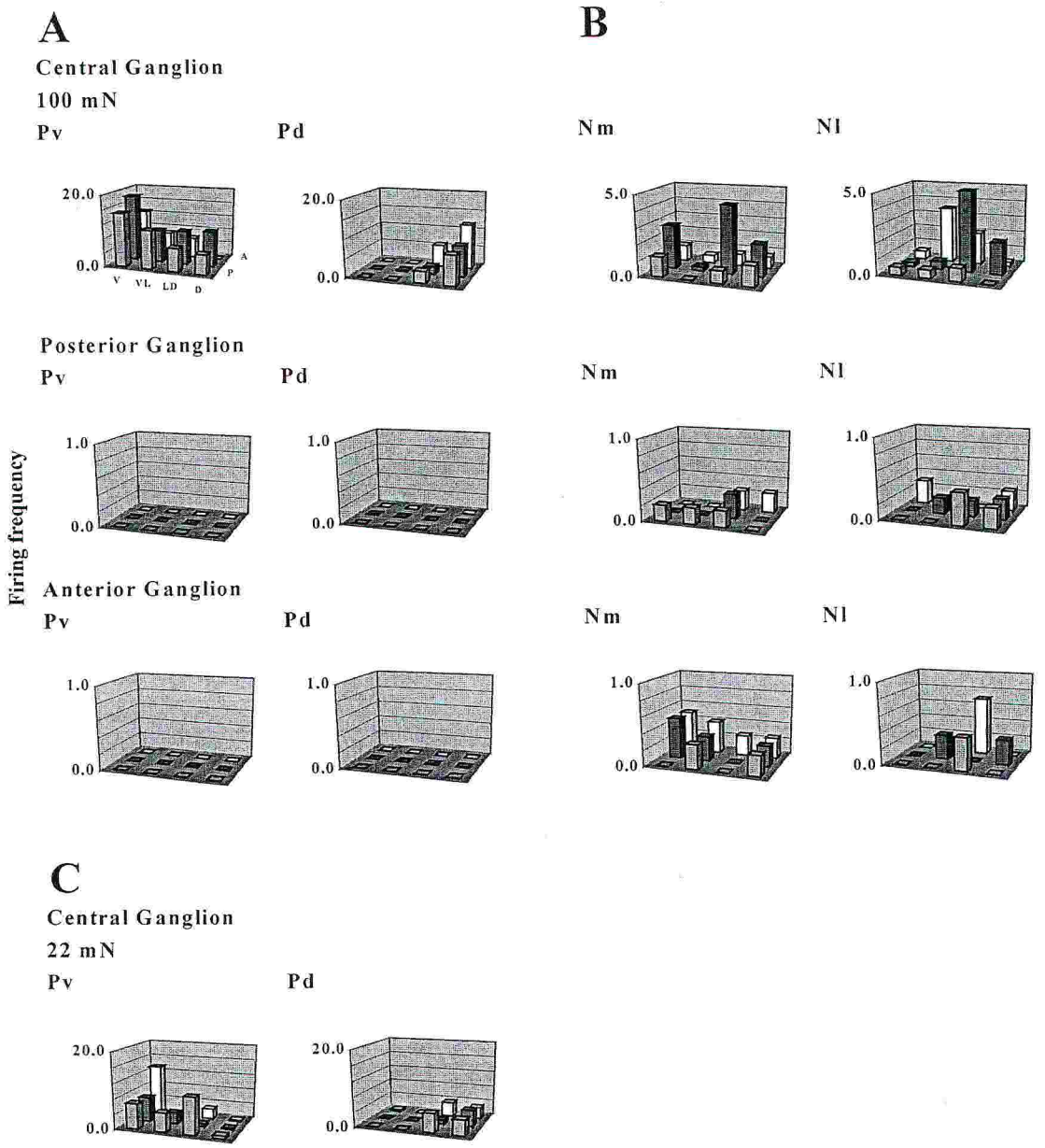

Fig. 3.8 Receptive fields for P and N cells during a prolonged mechanical stimulation. A and B: receptive fields for P (A) and N (B) cells of the central, posterior and anterior ganglion for a stimulation of 100 mN. C: receptive fields for P cells of the central ganglion for a stimulation of 22 mN. The receptive field was constructed by computing the average firing frequency during 1 s of stimulation. Eight stimulations were delivered at each location and action potentials in the time window of 1 s following the onset of the stimulation were considered. The stimulus was repeated every 50 s.

3.1.5 Mechanisms for adaptation of T and P cells

The different adaptation of P cells of the central and adjacent ganglia could be caused by a variety of biophysical mechanisms. First of all nerve endings in the central part and in the periphery of the receptive fields may have different properties, either in the endings themselves or in the mechanical milieu. Another possibility is that action potentials do not reach the cell body of P cells of adjacent ganglia because of conduction block (Van Essen, 1973; Yau, 1976; Macagno et al. 1987; Mar and Drapeau, 1996). In order to distinguish between these possibilities, intracellular recordings from P and N cells of the central ganglion and adjacent ganglia were performed during a prolonged stimulation. Panels A and B of Fig. 3.9 reproduce intracellular recordings from the body of a P_v and an N_m cell of the central ganglion, respectively, during a prolonged stimulation. The P_v and the N_m cell adapt little, and they fire action potentials at a rate of about 16 and 6 Hz respectively. Intracellular recordings from a P_v and an N_m cell of adjacent ganglia are shown in panels C and D respectively. The P_v cell fires four action potentials and then stops. The intracellular recording from the N_m cell shows an initial large action potential, followed by smaller depolarizing deflections, typical of conduction block (Van Essen, 1973; Macagno et al., 1987). Block occur when the action potentials conducted by fine axons of mechanosensory neurons fail to invade large axons and the soma because of the hyperpolarization of the cell with activity. This behavior was sometimes observed in N cells of adjacent ganglia, but never in P cells during a mechanical stimulation lasting 1 s. As shown in Fig. 3.9C, the intracellular recording from the P_v cell does not show conduction block (Yau, 1976). As a consequence, conduction block does not seem to be the major biophysical mechanism underlying adaptation in P cells of adjacent ganglia.

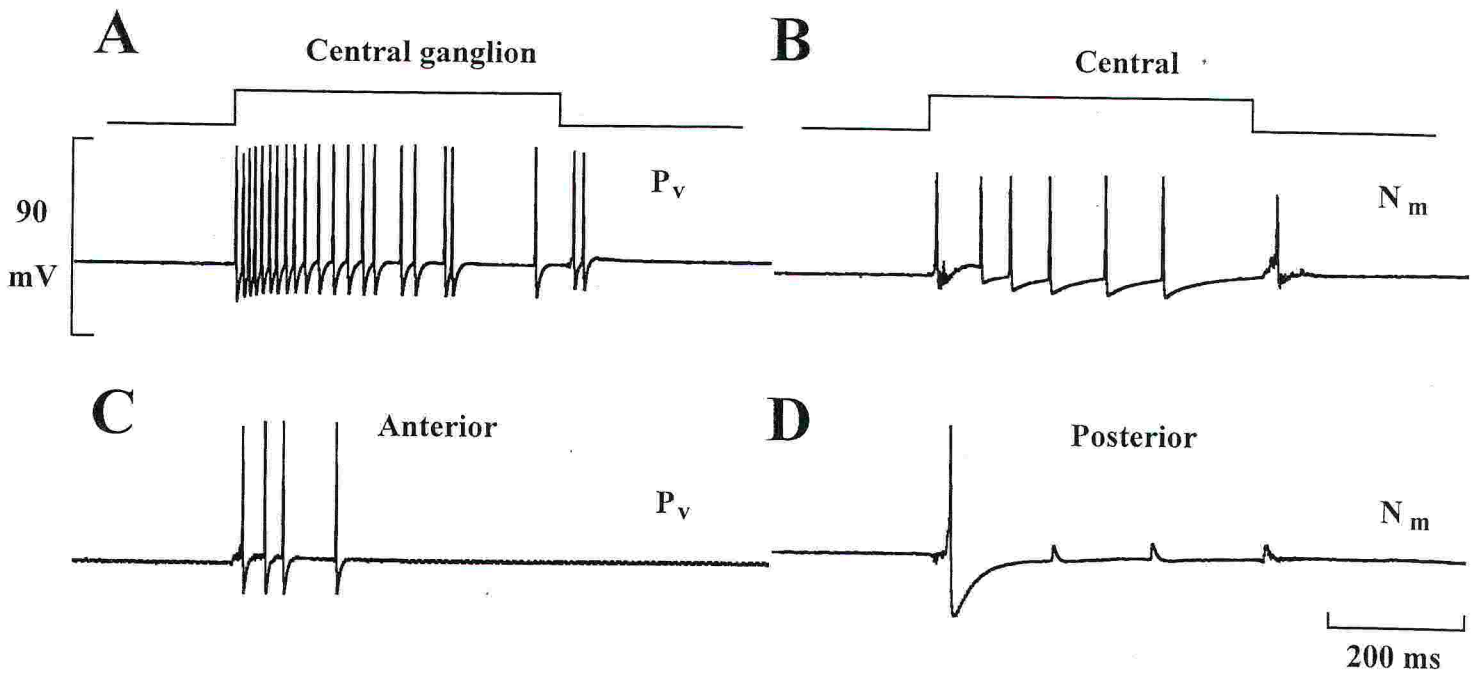

Fig. 3.9 Intracellular recordings during a prolonged mechanical stimulation. A and B: recordings from a P_v cell and an N_m cell respectively from the central ganglion. C and D: recordings from a P_v cell (anterior ganglion) and an N_m cell (posterior ganglion) during steady stimulation lasting 1 s as represented by the top bar. Stimulus intensity was 100 mN.

3.1.6 Responses to two stimuli

Biophysical mechanisms underlying adaptation of adjacent P cells can be investigated with experiments with two mechanical stimuli. Stimuli 1 and 2 were applied to two different locations, L_1 and L_2, of the receptive field, so that they activated the same P cell of an adjacent ganglion but a different set of nerve endings. Stimulus 1 was applied for 1 s in L_1 so as to induce a rapid adaptation of the P cell. When adaptation was fully reached, stimulus 2 was applied in L_2, so as to test the P cell sensitivity. The results of this experiment are shown in Fig. 3.10.

Panels A and B show extracellular recordings obtained by stimulating the two areas indicated in the insets. P cells of the central ganglion did not fully adapt, while those of adjacent ganglia quickly adapted. When the two stimuli were delivered simultaneously, the second mechanical stimulation was able to induce action potentials also in those neurons which adapted to the previous stimulation, as shown in Fig. 3.10C. Intracellular recordings for the three stimulations used in A, B and C are shown in panels D, E and F respectively. These recordings were obtained from P_d, N_1 and T_1 cells, from top to bottom, of the posterior ganglion (top and middle traces) and the central ganglion (bottom trace). In all three cells the second stimulation was able to produce action potentials, even after the cell adapted to the previous prolonged stimulation. This result indicates that the rapid adaptation observed in P and N cells of adjacent ganglia and in T cells of the central ganglion is likely to originate from properties of nerve endings and not from conduction block or membrane properties of the cell body.

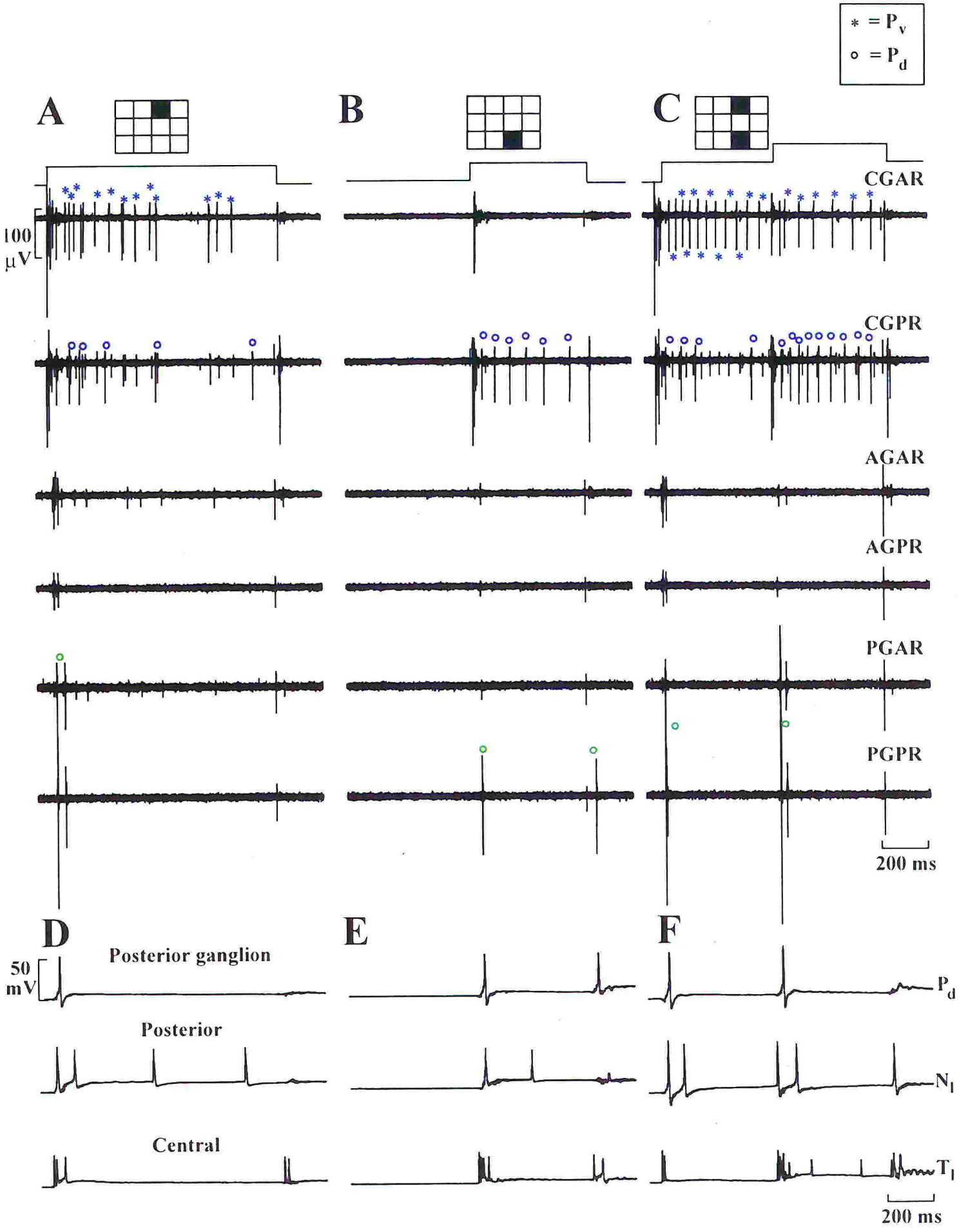
* = P_v
o = P_d
A
B
C
100 µV
CGAR
CGPR
AGAR
AGPR
PGAR
PGPR
200 ms
D
E
F
50 mV
Posterior ganglion
Posterior
Central
P_d
N_1
T_1
200 ms

Fig. 3.10 Responses to two stimulations in different areas and at different times. A and B: extracellular recordings showing responses to mechanical stimulation of 1 s (A) and 0.5 s (B). C: extracellular recordings obtained when the two stimulations used in A and B were delivered simultaneously. Labels beside the traces as in Fig. 2.1. D, E and F: intracellular recordings obtained during the same stimulation used in A, B and C respectively from a P_d and N_l cells of the posterior ganglion and from a T_l cell of the central ganglion. Stimulus intensity was 100 mN. Top bar indicates stimulus duration. Action potentials from identified mechanosensory neurons are labeled as in Fig. 3.2.

3.2 Discussion

3.2.1 Coding of brief stimuli

When the skin is briefly touched with a filament with a diameter of 0.1 mm exerting a weak force (less than 6 mN), between two and four T cells of the central and adjacent ganglia fire action potentials (see Fig. 3.4) and rarely it is possible to evoke action potentials only from T cells of the central ganglion. This is also a direct confirmation of the notion that the receptive field of T cells, obtained with a single electrode, extends beyond the respective body segment (Yau, 1976). These results show that receptive fields extensively superimpose with no gaps or interruptions (see Figs. 3.4 and 3.8) and that the sensitivity of the cells to mechanical stimulation in their major and minor receptive fields is similar.

In agreement with previous studies (Nicholls and Baylor, 1968) when the mechanical stimulation is increased above 20 mN, P cells in three ganglia are also stimulated. During the initial 100 ms the number of action potentials of T cells from the anterior and posterior ganglia were 23 ± 5 % and 32 ± 7 % of those recorded from the central ganglion. For P cells these values were 16 ± 5 % and 18 ± 7 % respectively. The ratio between the number of action potentials from mechanosensory neurons in adjacent ganglia and in the central ganglion was 0.55 and 0.33 for T and P cells respectively. Thus the electrical activity of adjacent ganglia is not negligible and is comparable in terms of number of evoked action potentials to that of the central ganglion. The onset of a mechanical stimulation of medium strength, i.e. about 20 mN, is detected in the leech nervous system by T and P cells of three ganglia and involves the activation of six to

fifteen different mechanosensory neurons. For even stronger stimulations, corresponding to about 50 mN N cells also respond to the stimulation (Nicholls and Baylor, 1968) and in these conditions between ten and twenty mechanosensory neurons code the mechanical stimulation.

Action potentials evoked by a mechanical stimulation occur with a delay, does not depend on the stimulus strength (see Figs. 3.5 and 3.6). This behavior is different from what observed in invertebrate photoreceptors (Fuortes and Yeandle, 1964) and olfactory sensory neurons (Reisert and Matthews, 1999), where the response latency is reduced in the presence of a stronger stimulation. As observed in Fig. 3.2, the first action potentials evoked in T and P cells appear at very reproducible times in different trials with little variability. Also this feature is not seen in photoreceptors and olfactory sensory neurons where highly variable bumps and events are observed (Menini et al., 1995).

For any mechanical stimulation T cells are the first mechanosensory neurons to be activated. After a delay varying between 15 and 20 ms P cells also fire action potentials. Action potentials in N cells are produced with a longer delay with respect to T cells, varying between 20 and 50 ms. This different delay in producing action potentials is likely to originate from different mechanisms. First of all nerve endings of T cells are the most superficial and the first to be stimulated when the skin is touched. In addition the transduction machinery may not have the same kinetics in different mechanosensory neurons.

3.2.2 Coding of prolonged stimuli

In the presence of a continuous mechanical pressure, T cells of the three ganglia and P cells of adjacent ganglia usually stop firing action potentials 200 ms after the stimulus onset (see Figs. 3.8 and 3.9) and rapidly adapt. Therefore only P cells of the central ganglion and N cells of the three ganglia fire action potentials in the presence of steady mechanical stimulation. This result was observed in 11 preparations, which were tested in 12 different regions of the skin. In one preparation, however, a prolonged stimulation of the skin on two specific areas produced a sustained discharge also in P cells of adjacent ganglia.

Different mechanisms can produce the adaptation observed in P cells of adjacent ganglia. Adaptation can occur because of intrinsic properties of the voltage activated conductances responsible for action potential initiation. This mechanism, however, is very unlikely to operate in our conditions, because P cells do not fully adapt when stimulated in the receptive field of their body segment (see Fig. 3.9). Another possible mechanism leading to adaptation of action potentials initiated in the accessory receptive fields is conduction block (Baylor and Nicholls, 1969; Van Essen, 1973; Macagno et al. 1987). This mechanism was sometimes clearly observed in N cells (see Fig. 3.9D), where the first action potential initiated in the accessory field propagates into the cell body, but not those following it. In this case the long lasting hyperpolarization following the somatic action potential of an N cell leads to the conduction block of action potentials arriving from distant branches (see Fig. 3.9D). This behavior, however, was never observed in P cells (see Fig. 3.9C and Fig. 3.10D and E), where the first somatic action potential is not followed by smaller depolarizing waves, typical of conduction block.

The experiments described in Fig. 3.10 show that a P cell of an adjacent ganglion, having adapted to a prolonged mechanical stimulation of a given area of its receptive field, is able to fire action potentials in response to the stimulation of a different area of the receptive field. Therefore the mechanism leading to adaptation is likely to be confined to the nerve endings. The density of nerve endings in the central body segment is larger than those in the adjacent body segments and this may lead to a different response to a prolonged stimulation in the center or in the periphery of the receptive field. In this view the generator current produced during mechanotransduction at each nerve ending adapts at some extent, but the much larger density of nerve endings in the central body segment is able to induce a sustained discharge of action potentials. The lower density of nerve endings of adjacent ganglia is initially enough to produce action potentials in the cell body, but not when adaptation takes place at nerve endings

3.3 Conclusions

The present analysis of the responses of mechanosensory neurons to stimulation of the skin shows some new basic properties of sensory coding in the leech skin. At the onset of the stimulation many mechanosensory neurons from at least three adjacent ganglia fire action potentials and a massive electrical signal is produced, without a clear localization of the stimulated area of the skin. After about 200 ms, this massive electrical discharge stops and a clear localization of the stimulated area can be achieved by the precise coding of P cells of the central ganglion. Therefore sensory coding in the leech skin is designed so as to have an initial high sensitivity and a poor localization,

but within 200 ms a good localization is achieved by an appropriate gain control mechanism.

This time dependent contraction of the receptive field properties is reminiscent of lateral inhibition observed in the eye of many invertebrate species (Coleman and Renninger, 1974; Coleman, 1975) and in the retina (Dowling and Werblin, 1971; Werblin, 1974; Cook and Mc Raynolds, 1998). Similar mechanisms have been also observed in the cochlea (Brownell et al. 1985; Zhao & Sacchi, 1999). This dynamic control of sensitivity and localization seems a basic property of sensory transduction present across species and sensory modalities.

Concluding, the results obtained in the experiments discussed in the present chapter shed new light on the coding of mechanical stimulation in the leech nervous system, providing a simultaneous analysis of the electrical activity evoked in mechanosensory neurons by mechanical stimulation. Several properties of mechanosensory neurons here described could be inferred from previous single neuron recordings (Nicholls and Baylor, 1968; Yau, 1976), but the relative sensitivity, timing and adaptation of different mechanosensory neurons can be firmly established only by simultaneous recordings, such as those here described.

4 Statistical independence and neural computation

The first part of my PhD thesis studied the first stage of information processing in the leech CNS. The present chapter deals with a different stage, i.e. the information transfer from sensory neurons to motor neurons.

The structure of the leech segmental ganglion, as discussed in chapter 1, makes it particularly interesting for studying input-output relations. In fact the information coming in the CNS can be clearly monitored as well as the response of the system that is in turn directed to the periphery. The reason for the simple access to the information flowing toward the CNS and backwards has to be found in its anatomy. The leech ganglion communicates with the rest of the CNS and body wall by discrete nerves (roots and connectives), so by simple extracellular suction pipettes it is possible to perform parallel recording of many neurons firing in concert. Moreover the single segmental ganglion presents a certain degree of independence from the rest of the CNS, as is typical in segmented animals composed by the repetition of single units. With regard to of the circuits that control the neurobiological functions, some of those are repeated in each ganglion and are responsible for the control at the level of single segments (Kristan, 1982; Lockery and Kristan, 1990a-b), while others are distributed along the whole animal and determine the coordination between the various segments (Thompson and Stent, 1976a-b-c; Calabrese, 1977; Peterson and Calabrese, 1982;

Brodfuerer et al., 1995; Brodfuerer and Friesen, 1986; Grillner et al. 1991). This fact suggests to investigate dynamics relegated in the single segmental ganglion.

The topic addressed in this chapter focuses on the statistical properties of the responses of neurons to the introduction of a sensory input in the ganglion.

An input coming from the periphery to the CNS like an action potential induced in a mechanosensory neuron by a touch stimulus, causes the response of several neurons that send action potentials back, through the roots, to the periphery or through the connectives to the other ganglia. Most of these neurons are motor neurons innervating muscle fibers in the body wall (Stuart, 1970, Lewis and Kristan, 1998a-b-c). I decided to analyze those neurons responding to specific stimuli, whose activity could be recorded by extracellular suction pipettes or by sharp intracellular electrodes.

Since in this set of experiments I wanted to study reproducibility of the output responses, an artificial input was produced, with current injection, by inducing a mechanosensory neuron to fire one or more action potentials at a very precise timing. The main goal of this analysis was to investigate how statistical properties of activation of the neurons participating in the network can be recognized as functional for neural processing.

The action potentials generated by different neurons were identified by analyzing extracellular voltage signals as explained in section 2.2.1.

The spread of excitation initiated by the action potentials evoked in the mechanosensory neurons crossed one or several synapses (both chemical and electrical) before reaching motor neurons. This observation reveals that many neurons are involved in these responses, therefore sensory information at this stage seems to be represented as a

distributed process (Lockery and Kristan, 1990a-b; Lewis and Kristan, 1998a-b; Zecevic et al., 1994; Wu et al., 1994a-b).

The results I have obtained show not only that the electrical activity elicited by the stimulation of a single mechanosensory neuron is distributed among a large number of neurons but also that it is characterized by significant spatial and temporal fluctuations in the occurrence of evoked action potentials. Moreover, the analysis reveals also that action potentials of coactivated neurons, i.e. activated by the same sensory input, are poorly pairwise correlated and exhibit a significant statistical independence.

This statistical independence seems to be a general property of such a system and can be considered functional for neural processing. As it will be discussed later, the statistical independence may be useful to improve reliability when the electrical activity is pooled among coactivated neurons.

4.1 Results

The experimental procedure consisted in inducing a mechanosensory neuron in an isolated leech ganglion to fire a fixed number of action potentials with a very precise timing (less than one millisecond). The response was recorded by the set-up described in section 2.1.3 (see also Fig. 2.2). The same stimulus was repeated many times, allowing to collect many trials of the same response.

Extracellular voltage recordings (Fig. 2.3) were examined to identify action potentials produced by the firing of the same neuron. The analysis of the background noise allowed the choice of a suitable threshold (red line in Fig. 2.3A) and events exceeding it

(Fig. 2.3B) were analyzed. Various parameters were measured (see Fig. 2.3C). Action potentials from the same neuron were extracted by appropriate clustering in a given plane (see Fig. 2.3D). Usually three or four characteristic action potentials were identified in each extracellular recording (Fig. 2.3E). Many leech neurons have been identified anatomically and their function has been well established (Muller et al., 1981; Lockery and Kristan, 1990a-b). These neurons can be impaled under visual control with intracellular microelectrodes and their action potentials, either spontaneous or evoked, are easily seen.

As already mentioned, neurons in the leech ganglion have stereotypical arborizations, with axons projecting into specific roots, so that many of them have a signature which can be identified in extracellular recordings from suction pipettes (see Fig. 2.4A). For instance an action potential of the right annulus erector AE(R) motor neuron (first column in Fig. 2.4A) produced simultaneous signals in the controlateral roots. Similarly action potentials from the left anterior pagoda (AP(L)) cell and the left longitudinal (L(L)) motor neuron (second and fifth columns in Fig. 2.4A) were recognized by the occurrence of simultaneous signals from different roots. Other motor neurons such as the dorsal excitatory (DE or cell 3) or the ventral inhibitory (VI or cell 2) produced extracellular voltage signals in one specific root (see third, fourth and sixth columns in Fig. 2.4A). The size and shape of these extracellular signals, constituting the neuron signature, varied from preparation to preparation and in each experiment the exact signature was determined by impaling these neurons under visual control (see section 2.2.2).

When a right dorsal P cell (P_d(R)) was impaled and one action potential was evoked, the signature of some neurons was recognized in the extracellular recordings (see Fig.

2.4B). The action potential of the P_d(R) produced a large signal on the ipsilateral dorsal posterior root (indicated by the arrow) and four other motor neurons (L(L), DE(R), DE(L), VI(L)) were identified (Fig. 2.4B) from their extracellular signature (Fig. 2.4A). The analysis of extracellular voltage signals illustrated in Fig. 2.3 and discussed in section 2.2.1 sorted between 20 and 30 different shapes of action potentials, of which between 5 and 10 were reliably assigned to identified leech neurons (Retzius cells, S cell AP cells, AE cells, L motor neurons, DE motor neurons and VI motor neurons). In each root and connective fiber there are approximately 2500 different axons (Wilkinson and Coggeshall, 1975) 98% of which have a diameter of less than 1 μm therefore producing rather small extracellular action potentials. As a consequence action potentials of only few neurons can be detected and identified.

4.1.1 Spatio-temporal variability of the evoked electrical activity

By inserting an intracellular electrode under visual control in an identified mechanosensory neuron and by recording extracellular signals with suction pipettes the electrical recordings shown in Fig. 4.1 and Fig. 4.2 were obtained. In each panel of Fig. 4.1 three different trials are superimposed and shown in red, blue and green. The left lateral T cell (T_l(L)) was impaled and 1 (A) and 4 (B) action potentials were evoked by injecting a current pulse. Similarly, the right dorsal P cell (P_d(R)) cell was impaled and 1 (C) and 2 (D) action potentials were evoked by current injection.

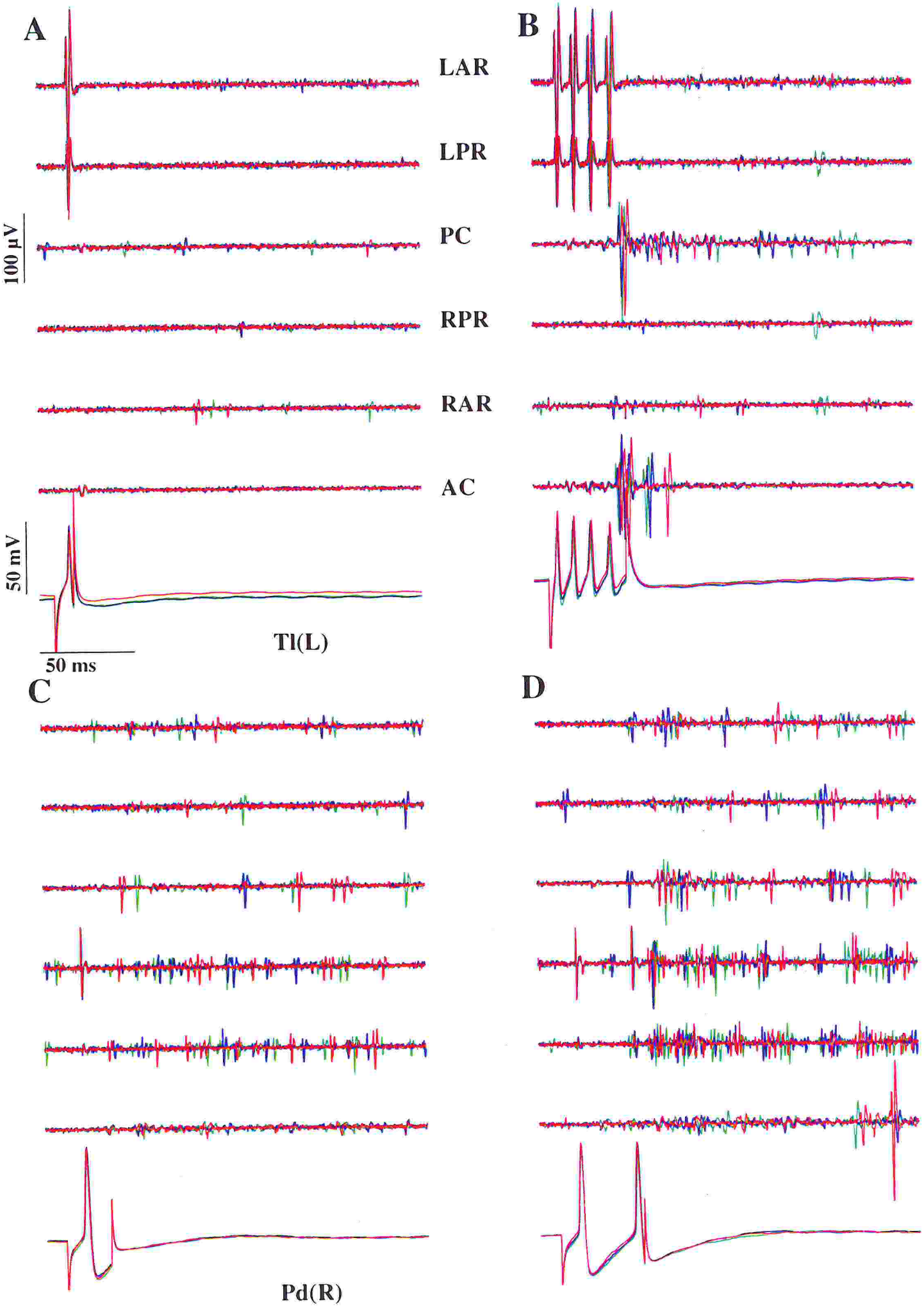
A
B
LAR
LPR
PC
RPR
RAR
AC
100 µV
50 mV
50 ms
Tl(L)
C
D
Pd(R)

Fig. 4.1 Jitter of the electrical response. In each panel recordings obtained from three different trials are shown in red, blue and green. From top to bottom 6 extracellular recordings are shown obtained from the left anterior root (LAR), the left posterior root (LPR), the posterior connective (PC), the right posterior root (RPR), the right anterior root (RAR) and the anterior connective (AC). The bottom trace is the intracellular recording from a T_I(L) cell (A and B) and from a P_d(R) cell (C and D). The evoked electrical activity recorded with suction pipettes increases with the number of spike elicited in the mechanosensory neuron. Observe the significant jitter of the evoked action potential recorded with the suction pipettes.

When the same current was repeatedly injected in a mechanosensory neuron (a T, a P or an N cell) every 30 seconds, the timing of the action potentials evoked in the same cell always occurred within 1 ms (see lower traces in the four panels of Fig. 4.1). On the contrary action potentials recorded in the roots and in the connectives, activated by synaptic inputs and not by current injection, were characterized by a large variability as shown by a simple visual inspection of the colored traces of Fig. 4.1. During different trials of the stimulation the timing of the same action potential could vary by even 50 ms and the jitter of the first evoked action potential had a standard deviation ranging from 5 to 30 ms. Besides observing a significant jitter, in different trials also different action potentials originating from distinct neurons appeared (Fig. 4.1). As a consequence the spread of excitation from a single mechanosensory neuron is characterized by a significant temporal and spatial variability. Analogously, Fig 4.2 shows ten trials not superimposed of the stimulation of a right ventral P cell recorded by a single extracellular electrode placed in the right posterior root. Even in this case it is possible to appreciate the spatio-temporal variability characterizing the action potentials.

4.1.2 First order statistics and characterization of temporal variability

The statistics of the responses has been studied. The first order statistics of the single neurons sorted was analyzed to characterize quantitatively the temporal variability. In order to clarify the first order statistical analysis, two particular cases of neurons activated by the stimulation of a P cell will be reported: the AP and the L cells which are known to be synaptically connected to the P cell (Nicholls and Purves, 1970; Macagno et al., 1987; Gu, 1991).

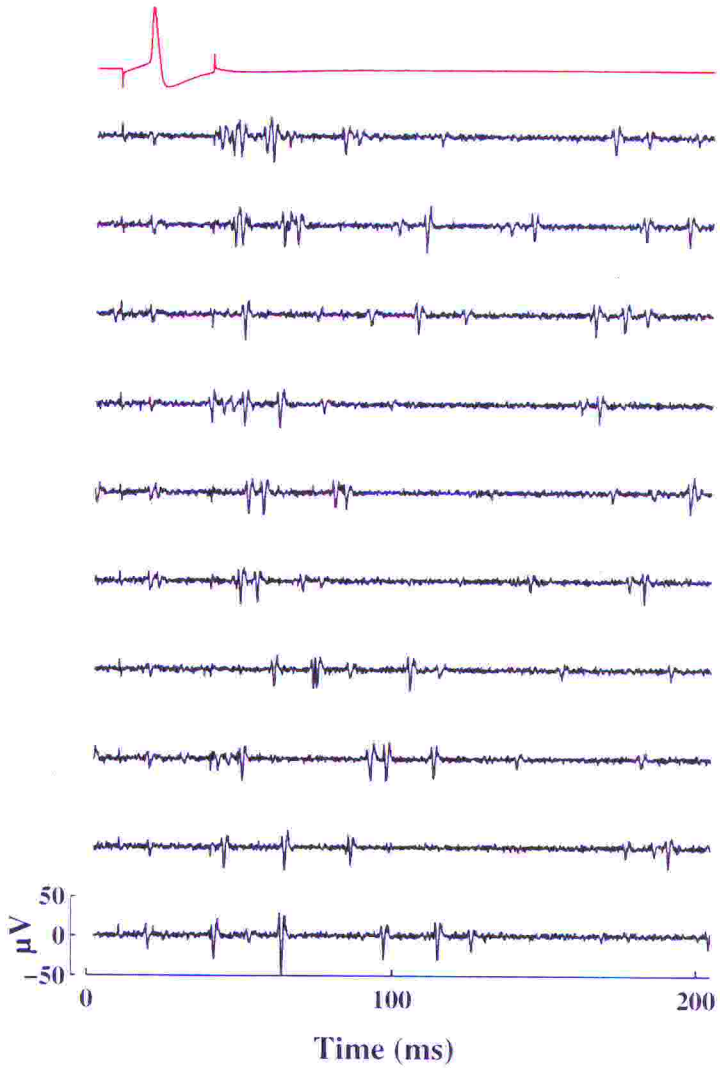

Fig. 4.2 Variability of electrical responses. Ten different trials recorded from the right posterior root after stimulating a right ventral P cell with an action potential. The intracellular recording of the P cell is reported in red on top of the traces (the small action potential corresponding to the stimulus, which is present in all traces, is the extracellular recording of the intracellular stimulation).

Fig. 4.3A-B illustrates double intracellular recordings from the AP(L) (upper traces in panels A and B) when one (Fig. 4.3A) and two action potentials (Fig. 4.3B) were evoked in the P_d(R) cell (lower traces in panels A and B). The large variability of the electrical response of the AP(L) (see Fig. 4.3A and 4.3B) can be characterized by analyzing the probabilistic structure of action potential occurrence. The probability of observing 0,1,2,3... action potentials within a time window of 200 ms following stimulation of the P cell with one or two action potentials is shown in Fig. 4.3C and 4.3E, respectively. When action potentials evoked in the right L motor neuron were considered (intracellular recordings not shown), the firing probabilities shown in Fig. 4.3D and 4.3F were obtained.

During different repetitions of the same stimulation, neurons could fire a variable number of action potentials from 0 to 4. This variability can be quantified by computing the entropy of the neuron.

Let us consider a neuron firing action potentials in response to a stimulus as a system with n different possible states represented by the numbers of action potentials produced in a given time window after the stimulus. The entropy, defined as

$$H = -\sum_{i=1}^{n} P_i \ln P_i$$

measures how the system occupies the states, where P_i is the probability of firing i spikes after the stimulus. If the system has the same probability to occupy all the states (maximum variability), $H=\ln n$, which is its maximum value. If, on the other hand, the system is not variable, i.e. the neuron responds each time in the same way, just a single state is possible with probability $P_i = P_1 = 1$. It follows that, in this case, $H = 0$.

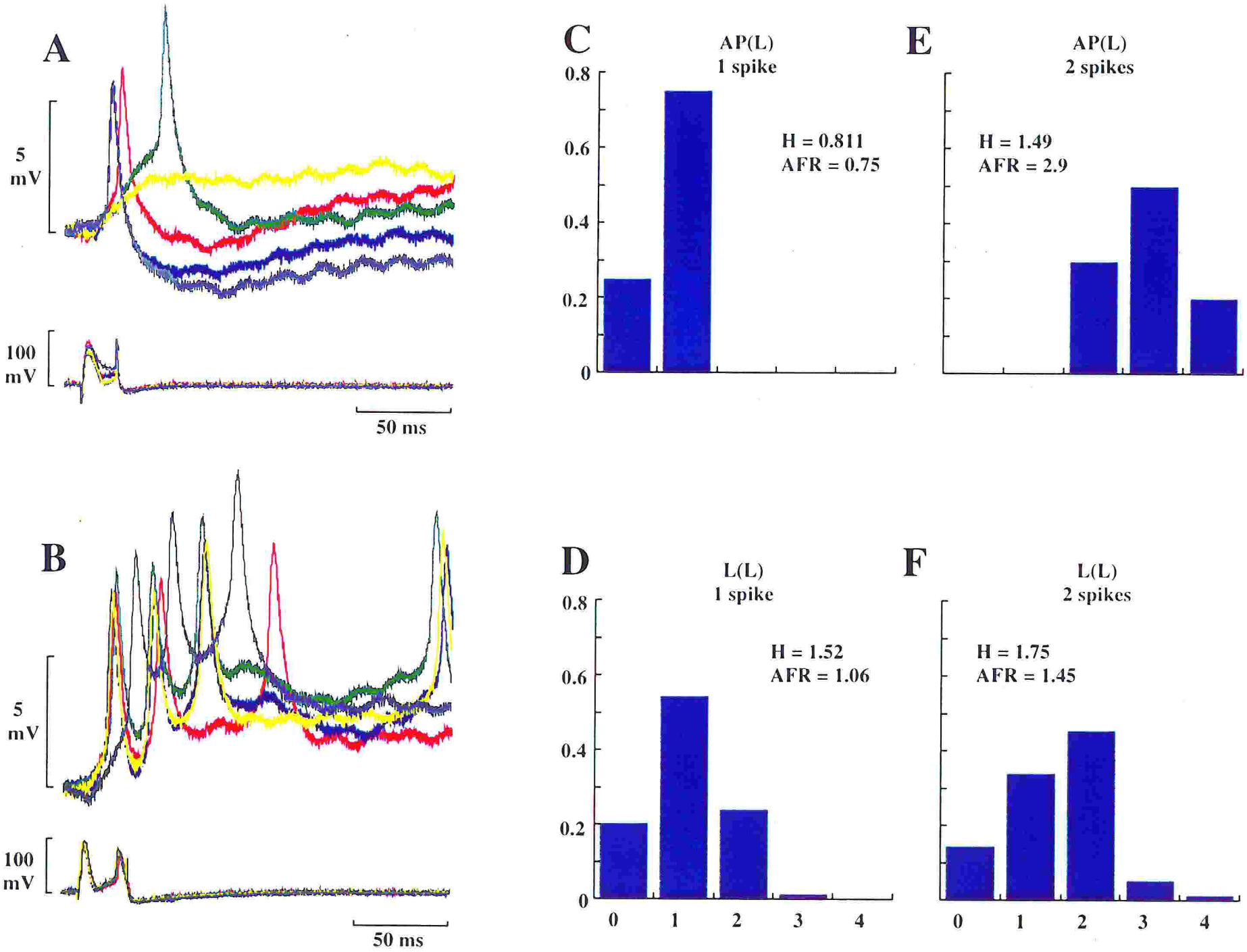
A
5
mV
100
mV
50 ms
B
5
mV
100
mV
50 ms
C
AP(L)
1 spike
H = 0.811
AFR = 0.75
0.8
0.6
0.4
0.2
0
E
AP(L)
2 spikes
H = 1.49
AFR = 2.9
D
L(L)
1 spike
H = 1.52
AFR = 1.06
0.8
0.6
0.4
0.2
0
0
1
2
3
4
F
L(L)
2 spikes
H = 1.75
AFR = 1.45
0
1
2
3
4

Fig. 4.3 Dependence of the action potentials statistics in an AP(L) and an L(L) motor neuron as a function of the stimulus intensity in a time window of 200 ms following the stimulation. A and B: Five superimposed intracellular recordings from the AP(L) when the P_d(R) cell was stimulated with 1 (A) and 2 (B) action potentials. Probability distributions of evoked action potentials in the AP(L) (C and E) and in the L(L) motor neuron (D and F) when the P_d(R) cell fired 1 (C and D) or 2 (E and F) action potentials. In this experiment the AP(L) and the P_d(R) cells were simultaneously impaled and 8 suction pipettes were used to record the extracellular activity from the connectives, the anterior roots, the dorsal and posterior posterior roots.

In the experiment analyzed in Fig. 4.3 and in the large majority of collected data the entropy varied between 1 and 2 bits, indicating the existence of at least three or four possible different states. Average firing rates of the two cells analyzed have been also reported in panels C-D-E-F of Fig. 4.3.

4.1.3 Second order statistics and correlation of electrical activity

The second order statistics of action potentials evoked by the activation of a single mechanosensory neuron was analyzed by computing joint probabilities and joint entropies (Figs. 4.4 and 4.5).

The firing of action potentials in identified coactivated neurons (DE(R), VI(L), L(L), DE(L) cells in Fig. 4.4) was consistent with a significant degree of statistical independence: if P_i (Pj) was the probability of observing at least one action potential in neuron N_i (N_j), the probability P_{ij} (red line) of observing at least one action potential in both neurons N_i and N_j was compared to $P_i * P_j$ (green line) as shown in Fig. 4.4A. Statistical methods have confirmed that the hypothesis that $P_{ij} = P_i * P_j$ can be accepted. This result was observed for almost every pair of neurons not electrically coupled and in all investigated ganglia.

A more complete way of verifying statistical independence, including all possible states for each pair of neurons, is to evaluate the relation $H_i + H_j = H_{ij}$, where H_i (H_j) is the entropy of the neuron N_i (N_j) and H_{ij} is the joint entropy of neurons N_i and N_j. When $H_i + H_j = H_{ij}$ the electrical activity of neurons N_i and N_j is statistically independent. Fig. 4.4B shows the comparison between the joint entropy H_{ij} and the sum of the two individual entropies $H_i + H_j$.

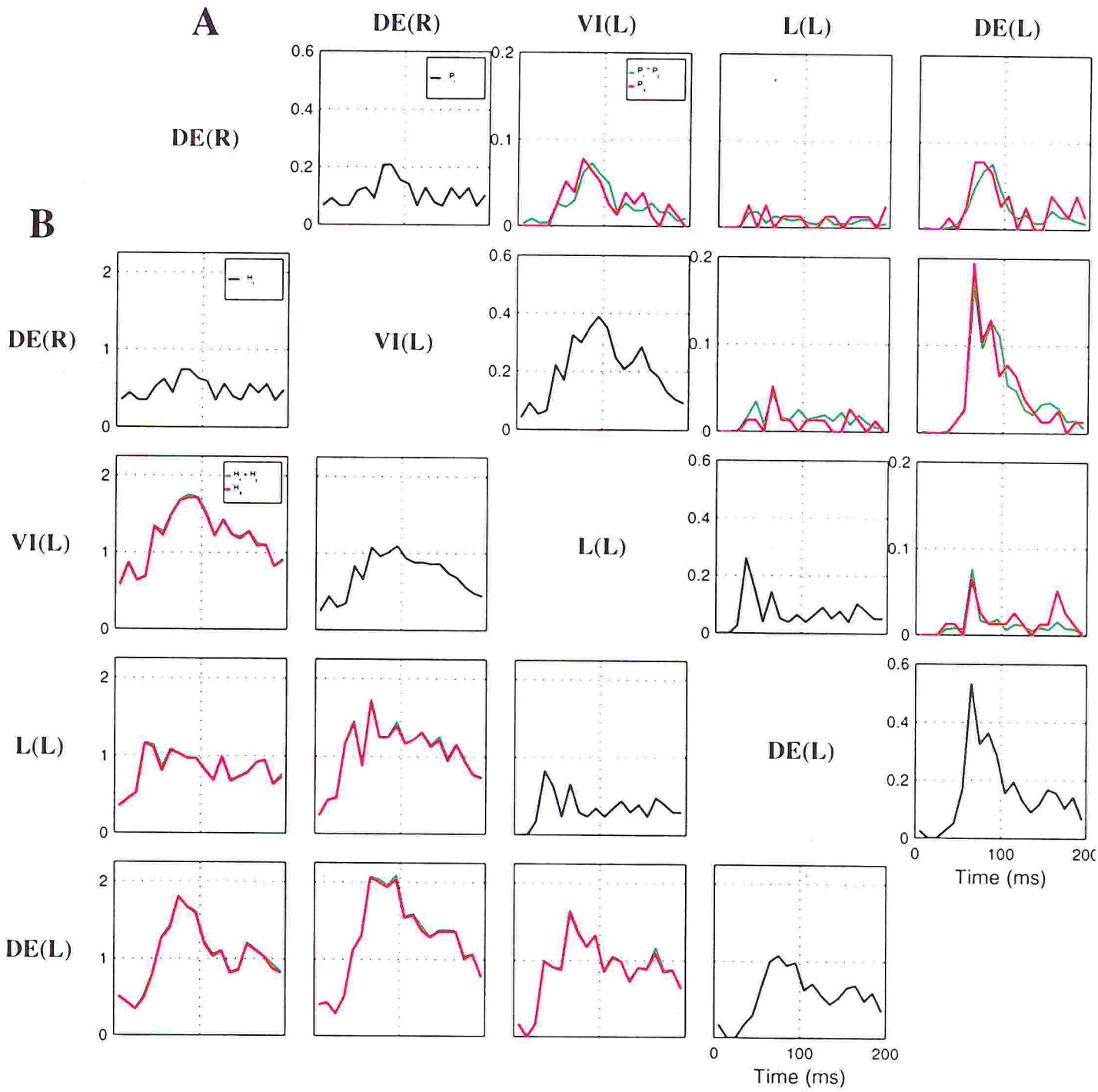
A
DE(R)
VI(L)
L(L)
DE(L)
DE(R)
B
DE(R)
VI(L)
VI(L)
L(L)
L(L)
DE(L)
DE(L)
Time (ms)
Time (ms)

Fig. 4.4 Joint statistics of pairs of identified coactivated neurons. A: In the upper diagonal panels the probabilities P_i of observing at least one action potential in four identified neurons N_1, N_2, N_3, N_4 versus time are reported. These four neurons were: DE(R), VI(L), L(L) and DE(L). The remaining 6 upper panels reproduce the probabilities P_{ij} (red lines) of observing at least one action potential in neuron N_i and one action potential in neuron N_j, and probabilities $P_i * P_j$ (green lines) for each combination of neurons N_1...N_4. B: In the lower diagonal panels the entropies H_i for neurons N_1...N_4 are reported. The remaining 6 lower panels reproduce the joint entropies H_{ij} (red lines) and $H_i + H_j$ (green lines) for each combination of neurons N_1...N_4. Probabilities computed over a time window Δt of 10 ms from 80 different trials of the same stimulation. In order to compare entropies and probabilities for the same pair of neurons, plots located symmetrically in the matrix have to be considered. Neurons were activated by the stimulation of the P_d(R) cell.

In the diagonal of Fig. 4.4B the individual entropies H_i for the four considered neurons are reproduced; in each other panel H_{ij} (red line) and $H_i + H_j$ (green line) are shown. These two quantities are equal for all considered pairs of neurons and for time windows from 10 to 50 ms.

From the electrical recordings obtained with suction pipettes it was possible to sort the shape of action potentials of a variety of neurons, activated by the stimulation of a mechanosensory neuron, which cannot be unequivocally identified, as those analyzed in Fig. 4.4. It is useful, however, to analyze the presence of statistical independence also among these coactivated not identified neurons.

Fig 4.5 reproduces the joint second order statistics for seven neurons activated by the stimulation of the P_d(R) cell. The statistical analysis was performed as in the case illustrated in Fig. 4.4.

For most neuron pairs considered, the relation $P_{ij} = P_i * P_j$ was satisfied with a level of confidence of 95%. The asterisks in Fig.4.5A indicate those cases in which the hypothesis could not be accepted. An intrinsic limitation to the analysis arises for pairs of neurons coactivated with small probabilities, comparable with the quantization error. In fact the probability P_i^{est} of observing at least one action potential in the neuron N_i in the time interval Δt was estimated from frequencies of occurrence n_i of action potentials in that time interval.

$$P_i^{est} = \frac{n_i}{N} \pm \frac{1}{\sqrt{N}} \sqrt{\frac{n_i}{N}\left(1 - \frac{n_i}{N}\right)}$$

where N is the total number of trials used for the estimation. For very small values of n_i, i.e. 1 or 2, P_i^{est} is affected by the quantization error $1/N$ which becomes predominant.

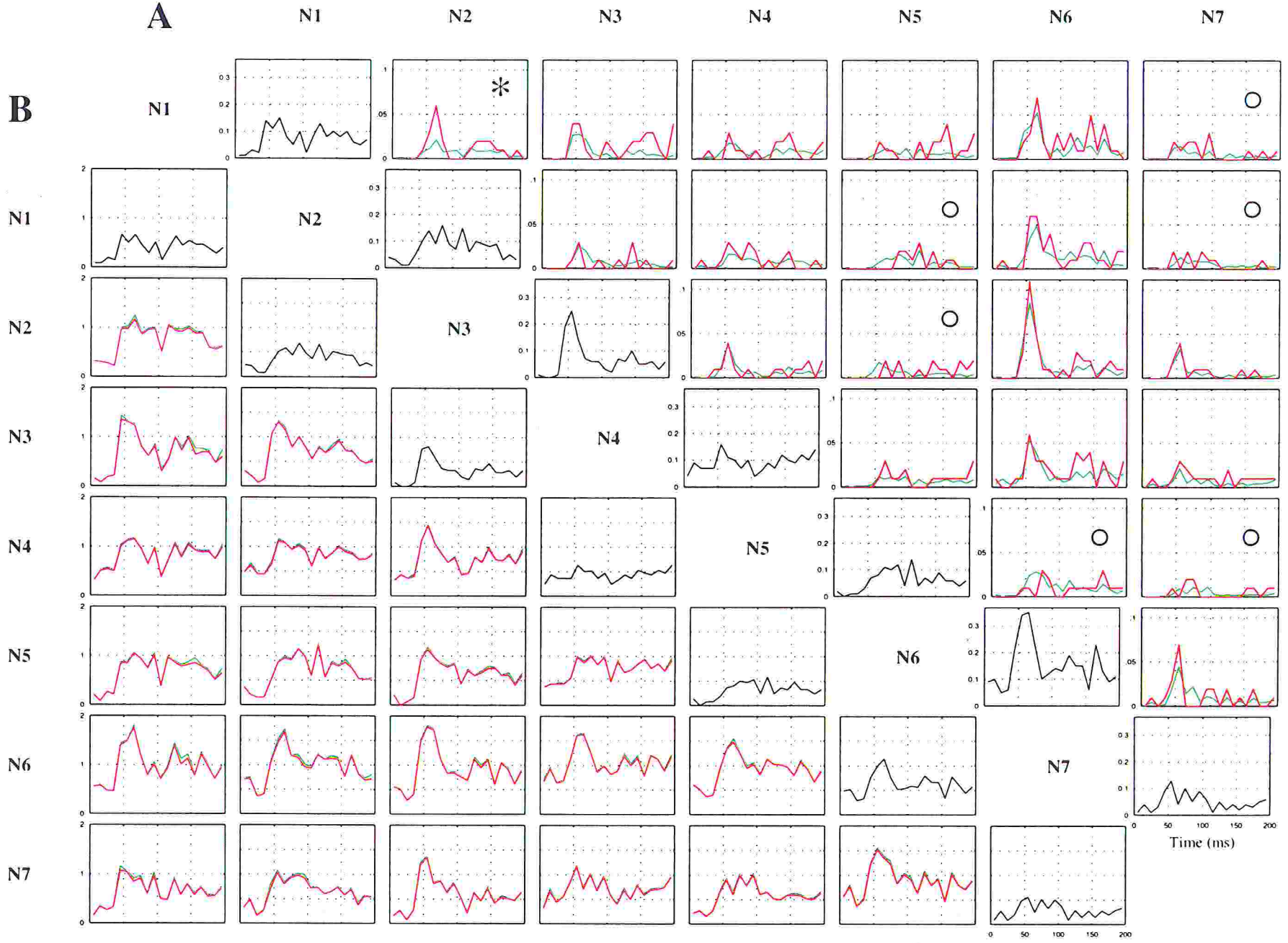
A
N1
N2
N3
N4
N5
N6
N7
B
N1
N2
N3
N4
N5
N6
N7
Time (ms)

Fig. 4.5 Joint statistics of pairs of coactivated neurons. Data from seven neurons. Neurons N_1, N_2, N_3 were identified in the left roots, N_4, N_5, N_6 in the right roots and N_7 in the anterior connective. A: In the upper diagonal panels the probabilities P_i of observing at least one action potential in neurons $N_1..N_7$. The remaining 21 upper panels reproduce the probabilities P_{ij} (red lines) of observing at least one action potential in neuron N_i and one action potential in neuron N_j, and probabilities $P_i * P_j$ (green lines) for each combination of neurons $N_1...N_7$. B: In the lower diagonal panels the entropies H_i for all neurons $N_1...N_7$ are reported. The remaining 21 lower panels reproduce the joint entropies H_{ij} (red lines) and $H_i + H_j$ (green lines) for each combination of neurons $N_1...N_7$. Asterisks indicate the pairs of neurons for which the hypothesis $P_{ij} = P_i * P_j$ cannot be accepted. Circles indicate cases in which analysis cannot be done because of the small values of P_{ij}, comparable with the quantization error.

The relation $P_{ij} = P_i * P_j$ was evaluated by testing the hypothesis that mean values were the same with a level of confidence of 95%. This hypothesis was tested in the time window in which P_{ij} was maximum for each pair of neurons. The histogram of the ratios between the mean values of $|P_{ij} - P_i * P_j|$ and their standard deviations for all pairs was also computed and these ratios were found to be distributed around the value 1 as expected (Lindgren, 1968). In these cases, indicated in Fig. 4.5A by circles, it was not possible to evaluate reliably statistical independence. For all pairs of neurons the joint entropy is equal to the sum of the two individual entropies. These results indicate that a large fraction of neurons activated by a mechanosensory neuron exhibits a significant statistical independence.

4.1.4 Distributed processing of sensory-motor information

Evidence for distributed processing of both sensory and motor information has been demonstrated in a large range of invertebrate and vertebrate species (Burrows, 1987; Shwartz and Kettner, 1988; Wu et al., 1994a-b; Georgopoulos, 1994, Georgopoulos, 1995) and in particular in the leech ganglion (Lockery and Kristan, 1990a-b; Lockery and Sejnowski, 1992). In the present work it has been also observed that, in agreement with previous studies, neurons responding to a sensory input constitute a distributed stochastic process. The results are shown in Fig. 4.6. This figure summarizes the electrical response of 7 selected neurons to 9 different stimuli. 3,4 and 5 action potentials evoked in T_l(R) cell, 1 and 2 action potentials evoked in P_v(R) and P_d(R) cells and 1 action potential evoked in N_m(R) and N_l(R) cells. These stimuli represent an effective spectrum of possible sensory inputs applied to the right side of a body segment

innervated by that ganglion. Each row illustrates the AFR evoked in a specific neuron by a given sensory stimulation. It is evident that every stimulation activates a different set of neurons and that almost every neuron receives an input from more than one mechanosensory neuron. Neuron labeled as N3 responded only to the stimulation of the $P_v(R)$ cell. The stimulation of the $P_d(R)$ cell appears to be coded by the activation of neurons labeled as N1 and N2 and the absence of activity in neuron labeled N5. Similarly the stimulation of the $T_I(R)$ cell activates several neurons (N1, N2 and N5) but not others (N3, N4, N6 and N7). Thus coding seems to be obtained by a distributed process, where the activation and silence (or inhibition) of specific neurons becomes the specific coding mechanism. In other words for each stimulus many motor neurons respond, and the same motor neurons respond to many input.

4.2 Discussion

The results presented in this chapter are consistent with the notion that neural computation in the leech ganglion is distributed (Lockery and Kristan, 1990a-b; Lewis and Kristan, 1998a-b; Baader and Kristan, 1995; Brodfuehrer et al., 1995) and demonstrate a significant spatial and temporal variability of this distributed processing: indeed electrical responses in neurons elicited by action potentials from a single mechanosensory neuron are characterized by significant spatial and temporal fluctuations. Temporal variability is associated to a large jitter in the occurrence of action potentials recorded extracellularly in the roots and in the connectives (see Figs. 4.1 and 4.2).

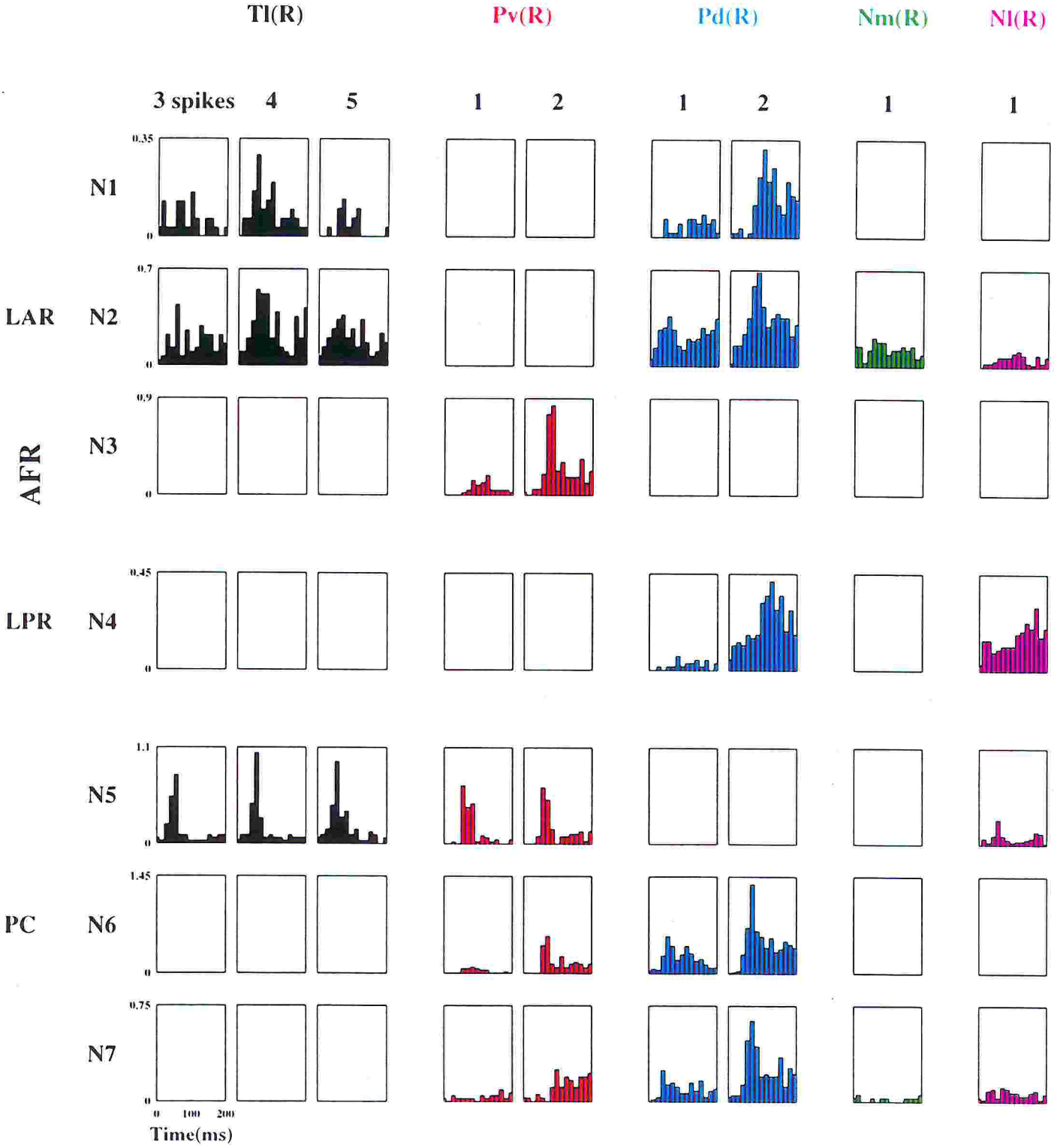
Tl(R)
Pv(R)
Pd(R)
Nm(R)
Nl(R)
3 spikes
4
5
1
2
1
2
1
1
N1
N2
N3
N4
N5
N6
N7
LAR
AFR
LPR
PC
0.35
0.7
0.9
0.45
1.1
1.45
0.75
0
100
200
Time(ms)

Fig.4.6 The divergence of different mechanosensory stimulations: AFR of 7 selected motor neurons and inter neurons to 9 different stimulations. 3,4 and 5 APs evoked in T_l(R) cell, 1 and 2 APs evoked in P_v(R) and P_d(R) cells and 1 AP evoked in the N_m(R) and N_l(R) cells. Each row illustrates the AFR evoked in a specific neuron (N1…N7) by a given sensory stimulation repeated at least 50 times every 30 seconds.

The spatial variability of the electrical responses was evident when the simultaneous activity of several neurons was observed. Indeed action potentials evoked in neurons by the stimulation of the same mechanosensory neuron were poorly pairwise correlated and exhibited a significant statistical independence (see Figs. 4.4 and 4.5). These results provide information on the nature of neural computation in the leech ganglion.

4.2.1 Origin of statistical independence

Recent reports have shown that the mechanism of action potential generation is very reliable (Mainen and Sejnowski, 1995), i.e. a suitable stimulation directly applied to the neuron can evoke spike trains with very reproducible pattern, while synapses exhibit in general high variability (Allen and Stevens, 1994; Larkman et al., 1997; Markram et al., 1997; Markram and Tsodyks, 1996; Wahl et al., 1995, Whal et al.,1997).

In the experiments here described the stimulus consisted in one or more action potentials induced in a mechanosensory neuron, activating through chemical and electrical synapses many different interneurons and motor neurons with a significant spatial and temporal variability. The large variability and the statistical independence of evoked action potentials here observed are likely to be due to the fact that the signal has to cross unreliable synapses, where action potentials have a low probability of crossing the synapse (Allen and Stevens, 1994), as often observed in the central nervous system (Markram and Tsodyks, 1996; Wahl et al., 1995, 1997; Zador, 1998; Hardingham and Larkman, 1998).

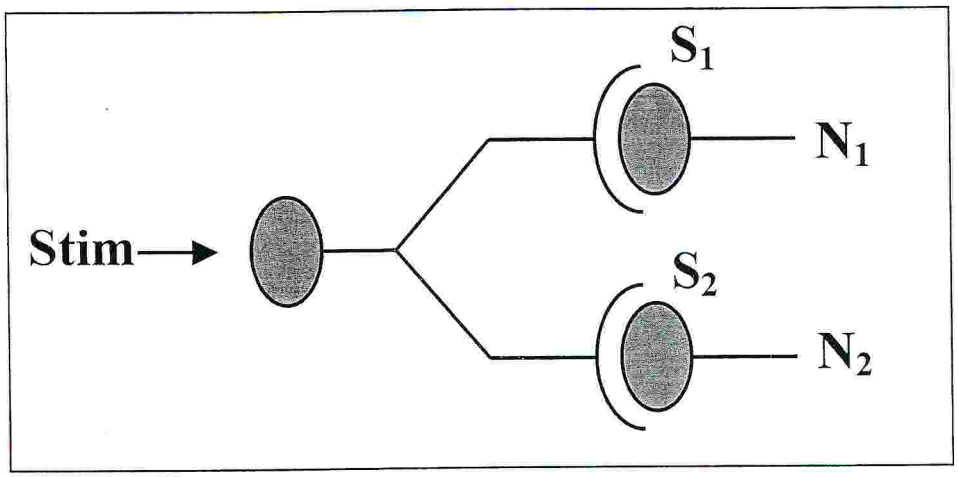

Fig. 4.7 Proposed origin of statistical independence. A simple scheme accounting for the variability of the electrical activity in a network. Synaptic release at the two terminals S1 and S2 of the same presynaptic neuron is statistically independent.

Let us consider a presynaptic neuron (see Fig. 4.7) projecting onto two different neurons N1 and N2, through chemical synapses S1 and S2 respectively. When an action potential in the presynaptic neuron invades the synaptic terminals, the release at the two synapses S1 and S2 is statistically independent, because S1 and S2 are physically distant and synaptic transmission is independent. Therefore failure and success in evoking a postsynaptic action potential in the two postsynaptic neurons are statistically independent. The scheme in Fig. 4.7 shows that action potentials of neurons activated by the same presynaptic neuron are statistically independent during their activation, i.e. their instantaneous firing probability $P_i(t)$ satisfies the relation $P_{ij}(t) = P_i(t) * P_j(t)$.

The scheme of electrical excitation represented in Fig. 4.7 is mediated by excitatory synapses, however excitation may also be mediated by disinhibition. The existence of the inhibitory pathways is consistent with the notion of statistical independence among coactivated neurons. This behavior is likely to occur in the majority of neuronal networks with chemical synapses.

4.2.2 Role of statistical independence

As the recordings from the leech ganglion (Fig. 2.2) show, applying a suitable stimulation the network results in an evoked pattern of activity which is distributed among a large number of neurons that individually respond in a not reproducible manner across the different trials. If a consistent output has to be produced, as the behavioral observations confirm (Lewis and Kristan, 1998), then the activity of groups of neurons has to be integrated. It will be demonstrated that the variability and the statistical independence in a distributed network can play a functional role to obtain a reproducible integrated response.

This integration can be computed in different ways, depending on how the outputs of the different neurons are combined in the network.

A simple measure which quantifies the variability of a single neuron in a given time window is the coefficient of variation (*CV*). If $x_i(r,s,t)$ is the number of spikes fired by neuron N_i during time window $(t, t+\Delta t)$ in response to trial r of stimulation s, the coefficient of variation $CV_i(s,t)$ can be defined as:

$$CV_i(s,t) = \frac{\sigma_i(s,t)}{AFR_i(s,t)}$$

where s is the applied stimulus, $AFR_i(s,t) = \frac{1}{R}\sum_r x_i(r,s,t)$ is the mean number of spikes (over the R trials of the same stimulation s) fired by neuron N_i in the time window $(t, t+\Delta t)$, and $\sigma_i(s,t) = \frac{1}{R}\sum_r \left(x_i(r,s,t) - AFR_i(s,t)\right)^2$ is the standard deviation.

This quantity must be compared to the coefficient of variation of the overall process. Two biologically plausible ways to achieve this are averaging and pooling.

Averaging. Suppose that the firing rate of each neuron N_i in response to stimulus s can be modeled as the realization of a stochastic process $\boldsymbol{x}_i(s,t)$. Suppose the network performs a sum of the neuronal activity of n distinct neurons $N_1 \ldots N_n$, converging onto a common target neuron and providing the final output $\boldsymbol{X}(s,t)$, a stochastic process which can be written as

$$\boldsymbol{X}(s,t) = \boldsymbol{x}_1(s,t) + \boldsymbol{x}_2(s,t) + \ldots + \boldsymbol{x}_n(s,t) \qquad (4.1)$$

The realizations $X(r,s,t)$ of this stochastic process are the spike counts (summed over all neurons) in the interval $(t, t+\Delta t)$ after trial r of stimulation s is applied: $X(r,s,t) = \sum_i x_i(r,s,t)$ where $x_i(r,s,t)$ is the activity of neuron N_i. The global coefficient of variation is simply defined as the coefficient of variation of $\boldsymbol{X}(s,t)$:

$$CV_X(s,t) = \frac{\sigma_X(s,t)}{AFR_X(s,t)} \qquad (4.2)$$

where $AFR_X(s,t) = \sum_i AFR_i(s,t)$ is the mean firing rate of $X(r,s,t)$, and $\sigma_X(s,t) = \sqrt{\frac{1}{R}\sum_{r=1}^{R}\left(X(r,s,t) - AFR_X(s,t)\right)^2}$ is its standard deviation.

Pooling. In the case of pooling, the stochastic processes x_i(s,t) are combined as components of the same vectorial stochastic process:

$$\Xi(s,t) = (\boldsymbol{x}_1(s,t), \boldsymbol{x}_2(s,t) \ldots \boldsymbol{x}_n(s,t)) \qquad (4.3)$$

In this case, the realizations of this stochastic processes consist of the outputs $x_i(r,s,t)$ of the isolated neurons N_i considered as components of a multivariate variable $X(r,s,t) = (x_1(r,s,t), x_2(r,s,t), \ldots x_n(r,s,t))$. Indicating as usual the average firing rate for neuron N_i as $AFR_i(s,t) = \frac{1}{R}\sum_r x_i(r,s,t)$, the coefficient of variation of the pooled output can be defined as

$$CV_{POOL}(s,t) = \frac{\sqrt{\sum_{i,j}\left|\Gamma_{i,j}(s,t)\right|}}{\sum_i AFR_i(s,t)} \qquad (4.4)$$

where $\sum_{i,j}\left|\Gamma_{i,j}(s,t)\right|$ is the sum of the absolute value of all the elements of the covariance matrix $\Gamma(s,t)$, defined as

$$\Gamma_{i,j}(s,t)=\frac{1}{R}\sum_{r=1}^{R}\left(x_i(r,s,t)-AFR_i(s,t)\right)\left(x_j(r,s,t)-AFR_j(s,t)\right)$$

Notice that the diagonal elements $\Gamma_{i,j}(s,t)$ of $\Gamma(s,t)$ are simply the variances $\sigma_i^{\,2}(s,t)$ of the firing rates $x_i(r,s,t)$ of neuron N_i. Also observe that $CV_X(s,t)$ can be written in terms of the elements $\Gamma_{i,j}(s,t)$ of the covariance matrix, giving $CV_x(s,t)=\sqrt{\sum_{i,j}\Gamma_{i,j}(s,t)}\Big/\sum_i AFR_i(s,t)$.

In section 4.1.3 it has been shown how the electrical activity of the different neurons in the leech ganglion is to a large degree statistically independent. It will now be shown with theoretical considerations how statistical independence allows to decrease variability in the electrical response of a network, for both the case where the outputs of different neurons is summed/averaged (Consequence 1) and the case where the outputs of different neurons are pooled (Consequence 2).

Consequence 1. If $\boldsymbol{x}_i(s,t)$ $(i=1, \ldots n)$ are statistically independent processes, and

$$\boldsymbol{X}(s,t)=\boldsymbol{x}_1(s,t)+\boldsymbol{x}_2(s,t)+\ldots+\boldsymbol{x}_n(s,t),$$

then the variance $\sigma_X{}^2(t)$ of $\boldsymbol{X}(t)$ is simply the sum of the variances of the individual processes (Papoulis, 1984):

$$\sigma_X{}^2(s,t) = \sum_i \sigma_i{}^2(s,t)$$

and the coefficient of variation of $\boldsymbol{X}(t)$ (Eq. 4.2) becomes:

$$CV_X(s,t) = \frac{\sqrt{\sum_i \sigma_i{}^2(s,t)}}{\sum_i AFR_i(s,t)} \qquad (4.5)$$

If $AFR_i(s,t) = AFR(s,t)$ and $\sigma_i(s,t) = \sigma(s,t)$ for all neurons N_i, and $CV(s,t) = \sigma(s,t)/AFR(s,t)$ is the coefficient of variation of the individual processes, then

$$CV_X(s,t) = \frac{\sqrt{N\sigma^2(s,t)}}{N \cdot AFR(s,t)} = \frac{1}{\sqrt{N}} CV(s,t)$$

which goes to 0 for very large N.

Consequence 1 states that by summing (or averaging, which brings exactly the same results) the individual responses of the different neurons a response is obtained which is asymptotically very stable, if the individual responses are statistically independent.

Observe that if, on the other hand, the stochastic processes $x_i(s,t)$ are highly correlated, e.g.

$$x_i(s,t) = x(s,t)\ i = 1,...N$$

variances will not add linearly, but as:

$$\sigma_X^{\,2}(s,t) = N^2\sigma^2(s,t)$$

and the coefficient of variation will be equal to the individual coefficient of variation:

$$CV_X(s,t) = CV(s,t)$$

regardless of the value of N.

Consequence 2. If $x_i(s,t)$ $(i=1, \ldots n)$ are statistically independent processes, and

$$\Xi(s,t) = (\boldsymbol{x}_1(s,t), \boldsymbol{x}_2(s,t) \ldots \boldsymbol{x}_n(s,t)),$$

then the covariance matrix $\Gamma(s,t)$ of the stochastic process $\Xi(s,t)$ is diagonal:

$$\Gamma(s,t) = \text{diag}(\Gamma_1^2(s,t), \Gamma_2^2(s,t), \ldots \Gamma_n^2(s,t))$$

and the coefficient of variation $CV_{POOL}(s,t)$ of Eq. (4.4) becomes

$$CV_{POOL}(s,t) = \frac{\sqrt{\sum_i \sigma_i^2(s,t)}}{\sum_i AFR_i(s,t)} \qquad (4.6)$$

which is exactly the same as Eq. (4.5): therefore $CV_X(s,t) = CV_{POOL}(s,t)$ for statistically independent processes; observe also that $\sum_i \sigma_i^2(s,t)$ the sum of the variances is simply the trace of Γ(s,t). As a consequence, also in this case, if all the processes $\mathbf{x}_i$(s,t) have the same mean AFR$_i$(s,t), the same variance $\sigma(s,t)$ and therefore the same coefficient of variation $CV(s,t) = \sigma(s,t)/AFR(s,t)$, $CV_{POOL}(s,t)$ asymptotically goes to 0 as $O\left(1/\sqrt{N}\right)$ as N increases:

$$CV_X(s,t) = \frac{1}{\sqrt{N}} CV(s,t).$$

Consequence 2 states that by pooling the individual responses of different neurons a response is obtained which is asymptotically very reproducible, if the individual responses are statistically independent. If, on the contrary, the stochastic processes $x_i(s,t)$ are highly correlated the global coefficient of variation will not necessarily go to zero. If, for example,

$$x_i(s,t) = x(s,t), \qquad i = 1,...N$$

then

$$\sum_{i,j}\left|\Gamma_{i,j}(s,t)\right| = N^2\sigma^2(s,t)$$

and the coefficient of variation will be equal as before to the individual coefficient of variation:

$$CV_X(s,t) = CV(s,t)$$

regardless of the value of N.

Finally, observe that it is always $CV_X(s,t) \le CV_{POOL}(s,t)$ (since $\Gamma_{i,i}(s,t) \le \left|\Gamma_{i,i}(s,t)\right|$) and $CV_{EXP}(s,t) \le CV_{POOL}(s,t)$ (since $\sum_{i\neq j}\left|\Gamma_{i,j}(s,t)\right| \ge 0$).

In order to confirm these theoretical considerations, Fig. 4.8 illustrates the behavior of the individual coefficients of variation as a function of time and compares them to the global coefficient of variation of the whole network. The global coefficient of variation is computed in the two ways specified by Eq. 4.2 and Eq. 4.4. It immediately appears that in our results the global coefficients of variation $CV_X(s,t)$ and $CV_{POOL}(s,t)$ are lower than the coefficients of variation $CV_i(s,t)$ of the individual neurons, as expected for statistically independent variables.

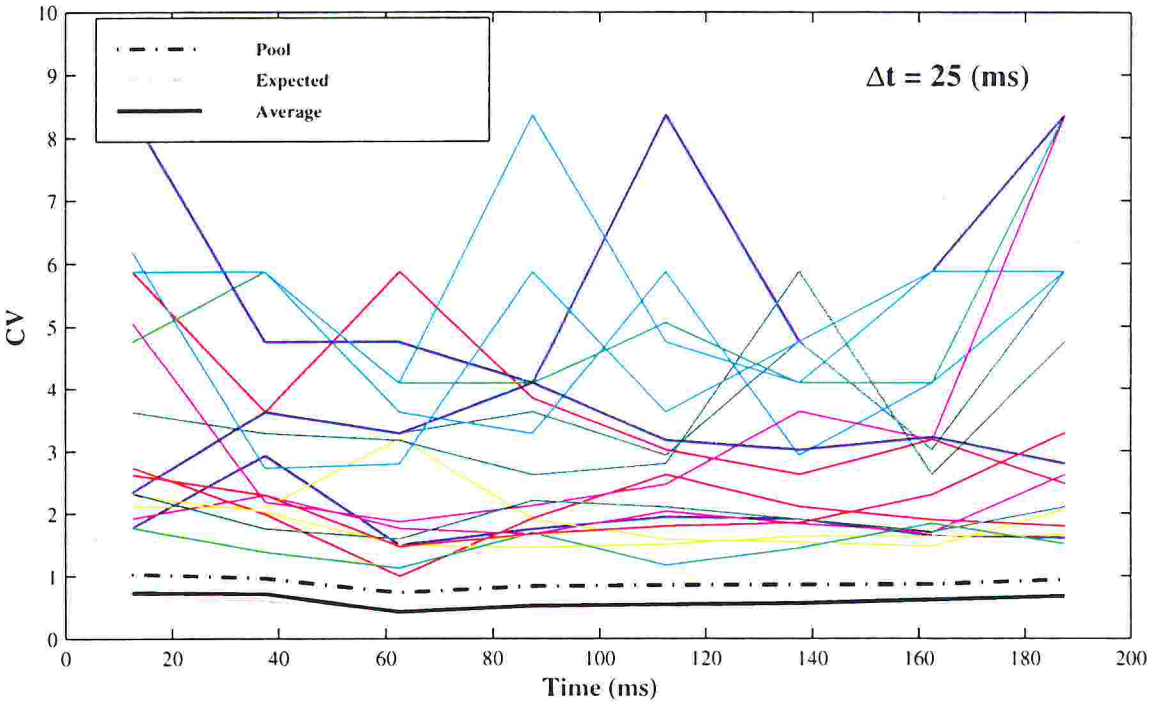

Fig. 4.8 Coefficient of variation as a function time of the individual neurons compared to the coefficient of variation (as a function of time) of the overall activity of the neuronal assembly in the leech ganglion. Coefficient of variation of different neurons whose activity was isolated in the recordings from the six suction pipettes (colored lines), compared with the global coefficients of variation $CV_X(s,t)$ ('Average', black line), $CV_{POOL}(s,t)$ ('Pool', black dotted line) and with $CV_{EXP}(s,t)$ ('Expected', gray line), which is the expected value of $CV_X(s,t)$ and $CV_{POOL}(s,t)$ (see Eq. 4.5 and 4.6) for statistically independent variables. Each coefficient of variation was computed every time window of 25 ms.

The value of the different coefficients of variation has been compared with the value of $CV_X(s,t)$ and $CV_{POOL}(s,t)$ which is expected for statistically independent variables, that is, with $CV_{EXP}(s,t) = \frac{\sqrt{\sum_i \sigma_i^2(s,t)}}{\sum_i AFR_i(s,t)}$ (see Eq. 4.5 and 4.6).

These results about the coefficient of variation are to be compared with the coefficient of variation computed for the case of spontaneous activity. For the leech, it was found that the coefficient of variation was in the order of $1/\sqrt{AFR}$ where AFR is the average firing rate, as predicted for a Poisson stochastic process.

I have therefore demonstrated that the statistical independence here described does not limit the performance of the nervous system. The same analysis was performed on a group of identified motor neurons. The CV of the evoked electrical activity of three motor neurons involved in bending reactions, i.e. the two DE motor neurons and the controlateral VI (colored lines in Fig. 4.9), was compared with the CV of the pooled activity of these three motor neurons (solid black line in Fig. 4.9) and the CV of the pooled activity of all neurons with action potentials recorded from the roots (dashed black line in Fig. 4.9). Pooling the electrical activity among only three neurons (solid black line) significantly reduced variability which was almost eliminated when a much larger neuronal population was considered (dashed black line). This result, observed in all examined ganglia, provides an important key to understand information processing and neural coding in the leech nervous system.

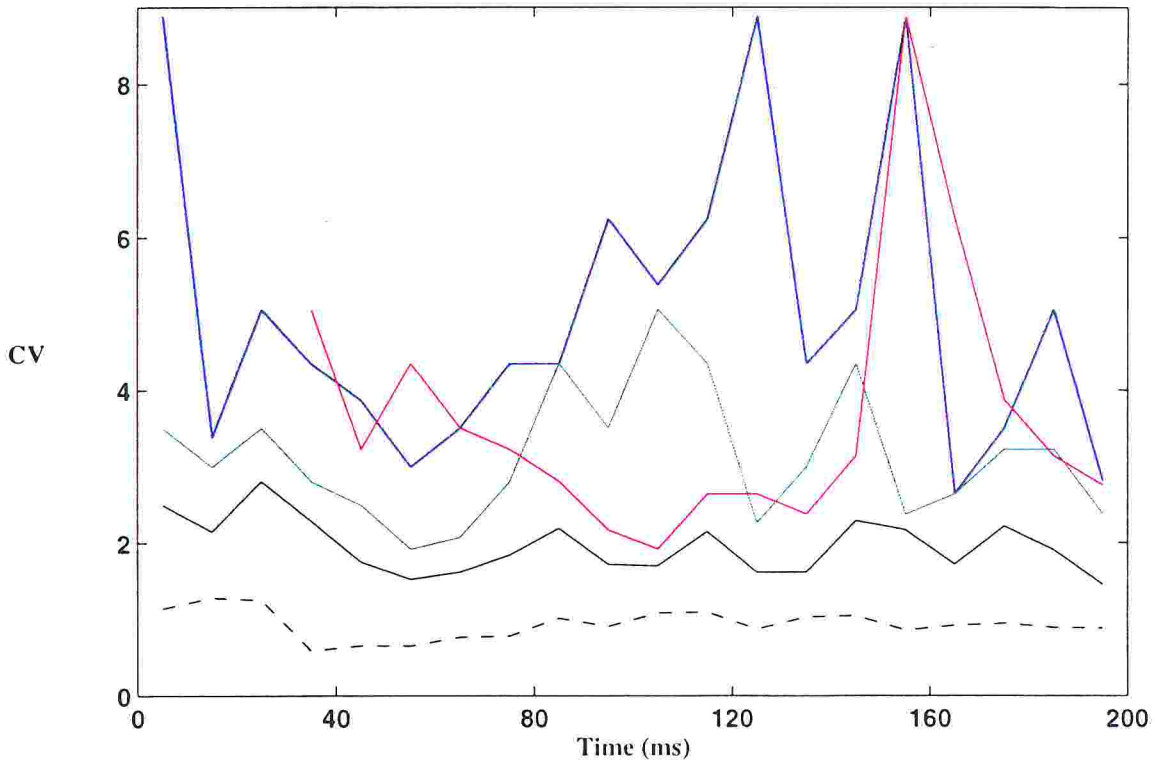

Fig. 4.9 Coefficient of variation as a function time of the individual identified motor neurons DE(L), DE(R) and VI(L) (colored lines) compared to the coefficient of variation of pooled activity of DE(L), DE(R) and VI(L) motor neurons (solid black line) and the pooled electrical activity from all neurons recorded from the roots (dashed black line). The P_d(R) cell was stimulated with 1 action potential. Data obtained from 80 different trials of the same stimulation.

Because sensory motor reactions and behavioral responses involve the simultaneous activation of many neurons, the large variability of the electrical activity seen in individual neurons (Figs. 4.1, 4.2 and 4.3) is reduced and almost eliminated when the electrical activity is pooled among a population of neurons (Figs. 4.8 and 4.9).

Reliability and reproducibility of important behavioral reactions of the leech, such as bending (Lockery and Kristan, 1990a-b; Lewis and Kristan, 1998a-b), involving the activation of motor neurons considered in Fig. 4.9, or swimming (Brodfuehrer et al., 1995) and crawling (Baader and Kristan, 1995) often initiated by the activation of a single mechanosensory neuron, may originate also from the neural mechanism here described, i.e. statistical independence among coactivated neurons. It is useful to observe that statistical independence and a poor correlation among coactivated neurons will not be observed in a neuronal network with reliable synapses. In this case action potentials cross synapses, the electrical activity is reproducible and the firing of coactivated neurons is correlated. For instance if neuron N_i makes a strong electrical synapse with neuron N_j, so that each time neuron N_i fires, an action potential is also observed in neuron N_j and $P_i = P_j = P_{ij}$, as observed with the two Retzius cells in leech ganglia (Hagiwara and Morita, 1962). When synapses are unreliable significant spatio-temporal fluctuations are observed and coactivated neurons exhibit statistical independence. In neuronal networks with unreliable synapses, reliability can be recovered by pooling the electrical activity among coactivated neurons. This mechanism, here described in the leech ganglion, may be a common feature of computation in neural networks with unreliable synapses.

4.3 Conclusions

This chapter has presented arguments supporting the view that neural computation can exploit distributed representations to achieve reliability. The experiments carried out, along with results obtained for other systems (Wu et al., 1994; Prechtl et al., 1997), show that different neuronal networks exhibit large spatio-temporal fluctuations in the electrical response to a given stimulus, due to the stochastic nature of synaptic transmission. I have demonstrated that systems subject to these fluctuations can still perform neural computation if the computation is distributed and the co-activated neurons are statistically independent.

Distributed processing can be realized in several ways. The two realizations investigated here are:

1) convergence on a common final neuron (Eq. 4.1): in this case fluctuations are efficiently removed by averaging synaptic input produced by statistically independent presynaptic neurons
2) distribution of the relevant neuronal signal among a population of neurons (pooling; see Eq. 4.3): the most important factor is the global electrical activity. This may be the case for sensory-motor reactions, where the output consists in the activation of many motoneurons, controlling a number of different muscles, as for crawling, bending and swimming of the leech.

The effects of distributed processing can be appreciated by measuring the trial-to-trial reproducibility of the electrical response of the network. This has been done by measuring the global coefficient of variation. The coefficient of variation of the overall electrical activity was found to approach zero as the number of computational elements increased, indicating that for large networks averaging or pooling provide viable means to perform reliable computation.

5 Conclusions and perspectives

Sensory coding of complex stimuli

From the results presented in chapter 3 it was shown that the simplest touch stimulus induces activity in several mechanosensory neurons with different dynamics of activation. The range of different stimuli to which the animal is subjected in a natural environment is wider than what was here considered. Parameters like direction, velocity, frequency of a given stimulus are very likely detected by the nervous system. How these tasks can be accomplished is still an open question. There are several possible explanations: the strategy can be found in the particular pattern of connections between neurons or in a distributed process like that observed in chapter 4; it may also be possible that part of the information is processed by single neurons or, finally, that all these mechanisms occur and are integrated. Using an experimental set-up and protocols as those previously described, the possibility of a distributed process underlying the detection of specific aspects of the stimulus can be investigated. A complex stimulus can be delivered onto the skin and parameters like direction, velocity or frequency can be controlled. The simultaneous recordings of sensory cells allow to determine mechanisms of population coding while the information about spread of action potentials in the different branches of neurons can be evaluated by considering the relative time delay in which action potentials are detected in the various recording sites.

Variability of motor responses

Chapter 4 demonstrated how reliability is achieved by the nervous system. So far the investigation has focused on the analysis of the responses of motor neurons. A natural development of this study is the evaluation of motor responses by monitoring muscles contraction. These experiments are now in progress; a system was set-up to record neural activity and to take simultaneously image sequences of the skin movements when a stimulus is delivered by evoking one or more action potentials in mechanosensory neurons. After checking the level of muscle contraction induced by a fixed number of action potentials evoked with intracellular electrodes in identified motor neurons, it is possible to evaluate if simple motor reactions in the leech segment recruit a massive activation of motor neurons, to estimate the duration of the process and finally to compare the variability in motor responses with respect to that observed in single motor neurons. The investigation of time courses of EPSP in motor neurons evoked by excitation of sensory cells is also a necessary step to understand input output relations in the leech ganglion.

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