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SYNCHRONICITY OF NOCICEPTIVE AND NON-NOCICEPTIVE ADJACENT NEURONS IN THE SPINAL DORSAL HORN OF THE RAT: STIMULUS-INDUCED PLASTICITY

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Abstract––Current knowledge of spinal processing of sensory information is largely based on single-cell recordings; however, temporal correlation of multiple cell discharges may play an important role in sensory encoding, and single electrode recordings of several neurons may provide insights into the functions of a neuronal network. The technique was applied to the lumbar spinal dorsal horn of pentobarbital-anaesthetized rats during background activity, steady-state noxious heat stimulation (48°C, 100 s), cold block spinalization or radiant heat-induced inflammation of the skin, and the recordings were evaluated by means of auto-correlation, autospectral and cross-correlation analysis. Background patterns obtained by these three methods were extremely stable in time. Autocorrelation with short lag peaks was observed in 72.2% of neurons (*n*=223). Background correlated discharges were found in 83.6% of the neuron pairs (*n*=134). Cross-correlation with a central peak, suggestive of common input to the recorded cells, was the most common pattern observed in almost all laminae and was associated with high incidence (91.8%) of overlapping receptive fields and with neurons with initial peak autocorrelation pattern. Cross-correlations with central trough were associated with increase autocorrelation patterns. Bilateral peaks in cross-correlation, suggestive of reverberating circuitry, were observed only for pairs of neurons located in laminae IV and V and were associated with rhythmic discharges in one or in both simultaneously-recorded neurons. Lagged peaks or troughs were observed in 4.6% and 2.2% of neuronal pairs, respectively. Long-lasting skin heating induced qualitative changes (pattern changes) in the cross-correlation of 21.6% of the neuron pairs and quantitative changes in 85.7% of them. During skin inflammation qualitative changes in the cross-correlation pattern were observed in 30.8% of the neuron pairs, and quantitative changes (strength and/or synchronization time) in about 57.7% of them. Spinalization induced quantitative changes in cross-correlation in the vast majority of neuron pairs.

The results of the present study suggest that discharges of neighbouring spinal dorsal horn neurons are strongly synchronized probably by propriospinal and primary afferent sources. The existence of functional reverberating circuitry was also evidenced. Finally, the functional synchronicity in the spinal dorsal horn presents stimulus-induced plasticity which consists mainly of changes on the strength and/or time of the synchronization and rarely of activation of new connectivities. Copyright © 1996 IBRO. Published by Elsevier Science Ltd.

Key words: neuronal network, sensory processing, cross-correlation, autocorrelation.

In contrast to the extensive body of morphological information about the neuronal network in the spinal dorsal horn (see Ref. 65 for a review), there is little known about the functional connectivity among its constituting neurons because research to date has been mostly carried out by means of single neuronal recordings. The introduction of multineuronal recordings (see Ref. 37 for a review) and the implementation of spike train analysis, e.g., autocorrelation[46] and cross-correlation,[45,47] have significantly advanced our understanding of the functioning of neuronal networks located in different parts of the CNS.[1,3,11,31] This type of approach can show discharge patterns, functional relationships and circuit interactions that cannot be characterized by single extra- or intracellular recordings.

Using these analytical methods on recordings from two different electrodes separated 1–1.5 mm, Brown et al.,[6] reported that about 12% of the neuronal pairs recorded in the lumbar spinal dorsal horn in the cat present causally discharge related; furthermore, all these discharge-related neurons present overlap in their cutaneous receptive fields. Intracellular recordings have shown that the information from primary afferents can activate multisynaptic pathways before activating the long projecting spinal dorsal horn neurons.[27,61] Probably spinal interneurons forming these multisynaptic pathways are able to delay (up to 20 ms) signals from primary afferents to

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supraspinally projecting neurons.[7] Pathophysiological conditions such as inflammation or strong noxious stimulation of the skin induce long-lasting changes in the cell excitability as well as in the extent and location of the receptive fields of spinal neurons.[29,44,68] Sensitization of cutaneous receptors as a result of local release of different neuromodulators, e.g., bradykinin and prostaglandin[49,69] is not sufficient to explain the expansion of the receptive fields toward the area of noxious stimulation as well as phenomena such as hyperalgesia and allodynia observed in chronic neuropathic or pathologic pain.[10] Furthermore, the effect of applying amino acids and peptides in spinal dorsal horn[22,32,49,69,70] and results from peripheral inflammation models[29] strongly support the notion that changes in the size of receptive fields are due to central mechanisms, probably an increase of excitatory inputs or a reduction of inhibitory inputs to the dorsal horn. Woolf and King[67] proposed the existence of 'relatively ineffective synapses' to some spinal dorsal horn interneurons to explain an area of 'low-probability firing' which surrounds the receptive fields. Activation of such synaptic contacts could cause the expansion of the receptive fields.

The functional connectivity of neighbouring neurons recorded at the same site in the spinal dorsal horn and the manner of sensory processing especially for nociception, remain obscure at the present. The presence of rhythmic discharging nociceptive neurons in the spinal dorsal horn and their role in the information transfer was recently postulated.[52] This rhythmicity could be induced by the functional connectivity within the spinal dorsal horn. Additionally, Sandkühler et al.,[53] described differential effects of spinalization on adjacent neurons in the lumbar dorsal horn, which supports the notions that neighbouring neurons can have different functions and can be under different modulatory influences. These experimental evidences could underlie integration function during signal processing in dorsal horn. On the other hand, mechanisms proposed to play a major role during signal processing, i.e. convergence, temporal and/or spatial summation and assembly coding depend on neuronal discharge synchronization. Therefore the study of functional networks in spinal cord under physiological conditions and under plasticity induced by their main inputs, i.e. the primary afferents and the propriospinal and supraspinal descending modulating systems, or under pathophysiological conditions such as long-lasting noxious stimulation or skin inflammation, should significantly contribute to our understanding of the structure and function of the spinal dorsal horn both under basal conditions and during phenomena such as chronic pain, hyperalgesia and allodynia.

The purpose of the present study is to characterize functional synchronicity among adjacent neurons recorded with the same microelectrode in the spinal dorsal horn both at baseline conditions and after changes induced by its main inputs. Some of the results have been published in abstract form.[13,14]

EXPERIMENTAL PROCEDURES

Anesthesia and surgery

89 male adult Sprague–Dawley rats bred at University of Heidelberg, 250–350 g body weight, were used. Anaesthesia was induced by 60 mg/kg pentobarbital sodium i.p. After loss of flexor, withdrawal and corneal reflexes, the trachea, the left jugular vein and the right carotid artery were cannulated to allow, respectively, mechanical ventilation with room air, if needed, fluid/drugs infusion, and monitoring of the arterial blood pressure. Pancuronium bromide (0.5 mg/kg) was given intravenously for muscle relaxation whenever artificial respiration was needed. To reduce secretion into the respiratory tract 10 mg of butylscopolamium bromide (Buscopan™) was given subcutaneously at the beginning of the experiment. Pentobarbital sodium was continuously infused intravenously (30–60 mg/kg/h) in glucose-tyrode solution in order to maintain a deep level of anaesthesia, as shown by stable mean arterial blood pressure (75–100 mmHg) and constant heart rate during noxious skin stimulation. Colorectal temperature was kept at $37.5 \pm 0.5°C$ (mean $\pm$ S.D.) by means of a feedback-controlled heating blanket.

The lumbar enlargement of the spinal cord was exposed by laminectomy from T11 to L4 for extracellular recording. Another laminectomy was made from C7 to T2 for reversible cold block. The vertebral column was firmly held in a horizontal position. For maximal recording stability, dorsal skin flaps were used to form a pool which was filled with 4–5% agar. A window was opened in the agar over the recording site and filled with warm mineral oil before the dura mater was opened. The left sural nerve was dissected free, mounted uncut on platinum hook electrodes for bipolar stimulation, and periodically bathed with paraffin oil. The left hind paw was fixed pad upwards with paraffin wax in a holder to allow noxious radiant heating of the glabrous skin. A local anaesthetic (Lidocaine 2%, Spray) was topically applied to surgical wound edges.

Recording and stimulation

Extracellular recordings were made with tungsten microelectrodes (4–5 MΩ at 1 kHz, A-M System, Inc.) driven by a microstep motor vertically into the left lumbar dorsal horn. Neurons with background activity were searched for and functionally classified.[23] Location and size of their mechanoreceptive fields were determined by means of a von Frey filament (6.7 g). To avoid electrode movement artefacts the data collection was started not earlier than 20–40 min after a neuron was found. Recordings were digitized by an A/D converter card DT2821-F-16SE at 32 kHz on a AT computer and stored on magnetic tape. Multineuronal activity was discriminated off-line into single neuron spike trains using the principal components method, based on the shape of waveform[51] (implemented by BrainWave Systems). This method uses simultaneously different shape parameters for discrimination, i.e. peak time, valley time, total amplitude, peak amplitude, valley amplitude, spike width, peak width and valley width, allowing an efficient spike discrimination, the quality of which was verified for every recording by visual inspection of spikes on screen. Only spikes with inflections on their rising phase, suggestive of initial segment or somato-dendritic activation were recorded. Cases which did not fulfill this criterion were extremely rare. Background activity was recorded for 5–40 min to allow collection of at least 2000, typically more than 5000, action potentials/neuron. The recordings were stored on magnetic

tape and on digital disk for off-line analysis. For neuronal characterization, the sural nerve was stimulated electrically either to recruit Aα and Aβ fibers only (0.1 ms, 2.5 V pulses) or to excite both A- and C-fibers (0.5 ms, 25 V pulses), and noxious radiant skin heating consisted of 50°C stimuli given for 10 s at intervals not shorter than 2 min. To study the neuronal response during the stationary phase of the heat-evoked response a 48°C, stimulus was given for 100 s. Skin inflammation was induced by radiant heat pulses of 48°C, four times for 100 s at intervals of 15 min. To avoid additive changes, once long-lasting skin heating or heat-induced skin inflammation was started, no further stimulation of receptive field was applied.

Spinalization and decerebration

A thermode (surface temperature 0°C) was placed directly on the spinal cord dorsum at the cervicothoracic junction. Consequently, mean arterial blood pressure dropped 40–50%, but always remained above 50 mmHg and this preserved the spinal blood flow.[26] Blood pressure returned to baseline values when the cold block was terminated. In some experiments a transection at the upper cervical cord was performed after local infiltration with 2% lidocaine. In another series of experiments the animals were decerebrated by the four-vessels occlusion technique[48] and then transected at the first cervical segment after local infiltration with 2% lidocaine. In these latter experiments recording was begun at least 4 h after terminating the administration of pentobarbital.

Histology

At the end of each experiment an electrolytic lesion (40 μA, 20 s) was made at the spinal recording site. The rats were sacrificed with an overdose of pentobarbital and transcardially perfused with phosphate-buffered saline followed by 4% formalin. Recording sites were identified in transverse sections of the spinal cord cut in a cryostat at 50 μm and stained with Cresyl Violet.

Data analysis

Data stored on FM tape and magnetic disk were analysed off-line. After discrimination of action potentials, and to warrant stationarity, individual neuronal discharge rates were evaluated for trends using the Mann–Whitney U-test[60] by which only discharge rates without trends at a statistical level of $p < 0.05$ were further analysed. To avoid bias in the selection for correlated pair of neurons, all simultaneously recorded spike trains were analysed after testing their stability. Only the stationarity periods of the recordings were analysed. The same statistical test was used for comparisons between the neuronal spike rate with the cord intact and that during spinalization. Differences in incidence were compared by means of the z-test.[50] Unless otherwise noted, data are presented as mean ± S.D. Levels of $p < 0.05$ are considered statistically significant.

Autocorrelation function of nth order interspike intervals was used to evaluate the temporal course of the neuronal discharge probability[46] and for autospectral analysis of spike trains (see below). Autocorrelation function was calculated for a total of 5000 bins (binwidth 1 ms) resulting 5 s of time window.

Spectral densities of stationary spike trains were estimated by autospectral analysis,[4,28,52] i.e. Fourier transformation of a total of 5000 bins from autocorrelation values. This method decomposes the autocorrelogram into an equivalent set of sine and cosine constitutive waves determining the spectral intensity at different frequencies with a resolution of 0.2 Hz. Raw autospectra were smoothed using a 20 term moving average. Significance of peaks against white noise on autospectra were tested by the Fisher kappa test[17] which determines the relative spectral density of a specific frequency against the total spectral density of the autospectrum.

Functional firing synchronicity among simultaneously-recorded neighbouring neurons was analysed by the cross-correlation method. Correlograms have a total of 1000 bins (binwidth 1 ms) within a time range of ±500 ms. Despite that *post hoc* examination of cross-correlation histograms suggest that visual evaluation picks out the appropriate category in over 98% of the cases,[16] two parameters were used to evaluate its features. First, the detectability index D[16,58] (maximal peak or through bin value divided by the standard deviation of bin values from the baseline). A D value greater than 2 indicates that a peak or trough does exist (non flat cross-correlation). Second, the strength index k[25,33,38,57] (maximal peak or trough bin value divided by the arithmetic mean of bin values from the baseline) which indicates the strength of the peak or trough. Values of k greater than 1 are considered to represent an excitatory relation and less than 1 an inhibitory one. Peak or trough width was determined at the baseline of the cross-correlograms.

Although the multineuronal single electrode recording is the most suitable method for the study of functional synchronicity of very close neurons, it is not possible to discriminate action potentials if they overlap in time,[21,64] thus a 1 ms delay time in cross-correlations constitutes an area not analysed in the present work.

RESULTS

Neurons sample and general recording conditions

Results were obtained from 119 pairs, three triplets and one quadruple of neurons for a total of 134 pairs of neuronal combinations. Only neurons with spikes which showed somatodendritic deflection indicating recording of the soma and stationary discharge rate were included in this analysis. From 176 units which were fully characterized, 38 (21.6%) were low threshold, 128 (72.7%) were multireceptive and 10 (5.7%) were nociceptive-specific neurons. The remaining neurons were not fully characterized and therefore not classified. The recording volume estimated by the method described by Gochin et al.,[21] for a single recording microelectrode, i.e. the mean of the distance traversed by the electrode between the appearance and the disappearance of the neuron's activity, was 100 ± 22 μm (mean ± S.D.). This diameter represents the possible maximal separation between simultaneously-recorded neurons.

Neurons with correlated discharges

Correlated background discharges were found in 112/134 (83.6%) pairs of neurons recorded with the cord intact. A central peak, i.e. cross-correlogram with peak straddling the zero time bin (Fig. 1B), was the most common pattern (Table 1). Representative cross-correlation patterns and histologically-verified recording sites in the dorsal horn are presented in Fig. 1. Flat cross-correlations were observed in laminae IV–VI (Fig. 1A). Cross-correlograms with central peaks or central troughs were observed in almost all laminae (Fig. 1B and C). In contrast, patterns with bilateral peaks were observed only in laminae IV and V (Fig. 1D). The stability of these cross-correlation patterns was corroborated by long-lasting recordings

Table 1. Cross-correlation patterns of background discharges in pairs (n=134) of spinal dorsal horn neurons recorded in the intact cord

Cross-correlation patterns	Incidence (%)		Incidence per lamina (%)							
			I	II	III	IV	V	VI	VII	VIII
Correlated	112	(83.6)								
Central peak	70	(52.2)	-	4.3	18.6	38.6	35.7	-	1.4	1.4
Central trough	21	(15.7)	4.8	9.5	28.6	33.3	23.8	-	-	-
Bilateral peak	7	(5.2)	-	-	-	42.9	57.1	-	-	-
Delayed peak	6	(4.6)	-	-	16.7	33.3	50.0	-	-	-
Central peak + delayed trough	5	(3.7)	-	-	-	40.0	40.0	20.0	-	-
Delayed trough	3	(2.2)	-	-	33.3	66.7	-	-	-	-
Uncorrelated	19	(14.2)	-	-	-	26.3	36.8	36.8	-	-
Unclassifiable	3	(2.2)	-	-	33.3	33.3	33.33	-	-	-

Incidence is reported as the number of found neuron pairs, percentage is presented in parentheses referred to the total neuronal pairs tested (n=134), lamina incidence is reported as percentage of the total number of every cross-correlation pattern. Triple neuron recordings and their possible neuronal pair combinations were also analysed; thus the total number of analysed neurons in pairs may exceed the absolute number of neurons. Unclassifiable patterns are those which could not be classified due to insufficient bins in the histogram.
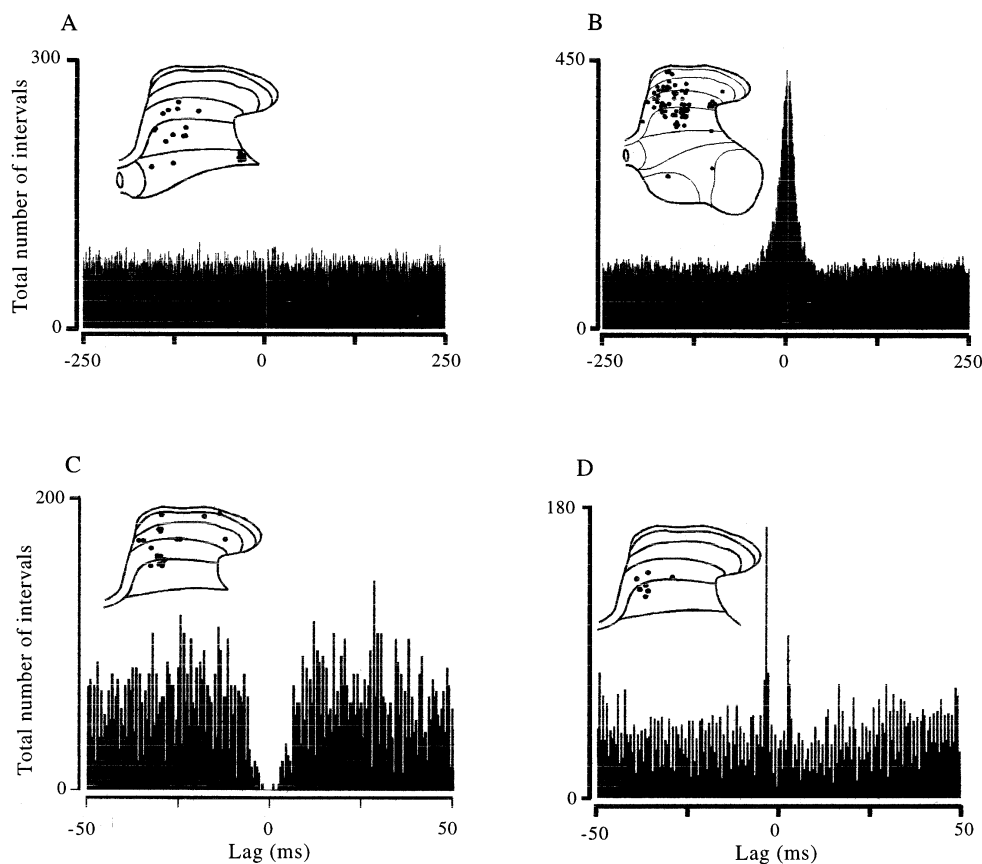

Fig. 1. (A–D). Typical examples of cross-correlograms of discharges of simultaneously recorded pairs of spinal dorsal horn neurons. The histologically verified recording sites where each cross-correlation pattern was recorded are presented in the inserts, as superimpositions on a representative section through the lumbar spinal dorsal horn.

(up to 10 h) from 37 pairs of neurons with correlated discharges. An example during background activity is presented in Fig. 2. Autocorrelation patterns were in the same way strongly stable in time.

The strength of the discharge correlation in neuron pairs (k index) for central peak correlograms was unrelated to depth of the recording (Fig. 3A). Indeed, k=9.9±9.7 for superficial laminae (<300 µm from
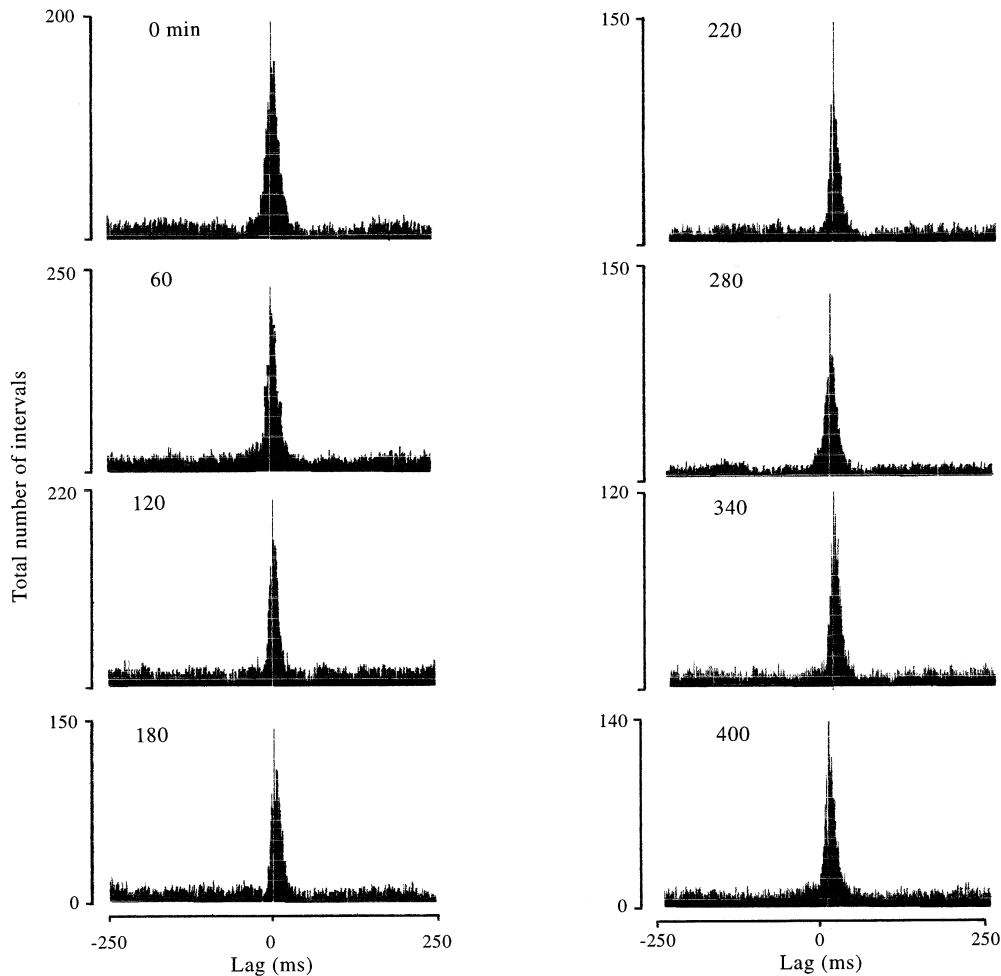
Fig. 2. Cross-correlograms of background discharges of two spinal dorsal horn neurons showing a pattern with a central peak which is suggestive of common input. Each cross-correlogram was calculated from 15 min recordings every 40–60 min during 7 h. Note the stability of the pattern along time.

cord dorsum, 119.6 ± 86.3 μm), which was not statistically different ($p>0.05$) from $k=6.03 \pm 7.2$ for deep laminae (300 μm, 865.4 ± 292.5 μm). However for pairs of neurons with central trough correlograms (Fig. 3B), the strength of the interaction in pairs located deeply was (0.321 ± 0.239) statistically lower ($p<0.05$) than that from pairs located superficially (0.2 ± 0.057). The base width of central peaks or central troughs (continuous range from 3 to 200 ms) was not related to depth of recording site ($p>0.05$). Ninety-one functionally-characterized neurons were analysed (Table 2). Incidence of cross-correlation patterns was not significantly different ($p>0.05$) between all pair combinations of functionally-classified neurons, i.e. two low-threshold, one low-threshold and one multireceptive or two multireceptive neurons.

Receptive fields and cross-correlation patterns

The excitatory mechanoreceptive fields of 83 neuron pairs were determined and classified as non-overlapping (11/83, 13.3%) and overlapping (72/83,

86.7%). Pairs of neurons with correlated discharges displayed a significantly higher incidence of overlapping receptive fields (65/72, 90.3%, $p<0.01$) than those not correlated (Table 3). Pairs of neurons with central peaks or with central troughs in the cross-correlograms presented a low incidence of non-overlapping receptive fields (6.1%) and high incidence of overlapping receptive fields (93.8%, $p<0.01$). Sizes of receptive fields from simultaneously-recorded neurons were not related to each other, as revealed by regression analysis ($r=0.21$, $p>0.05$).

Autocorrelation and cross-correlation patterns

Autocorrelation analysis of discharges from 223 neurons revealed the existence of two patterns, first, a peak probability to fire shortly after the refractory period of an action potential (Peak pattern, see Fig. 4A) which was found in 161 (72.1%) of the neurons distributed over all laminae (Fig. 4C) and second, a reduced probability to discharge following an action potential with a slow recovery of the excitability
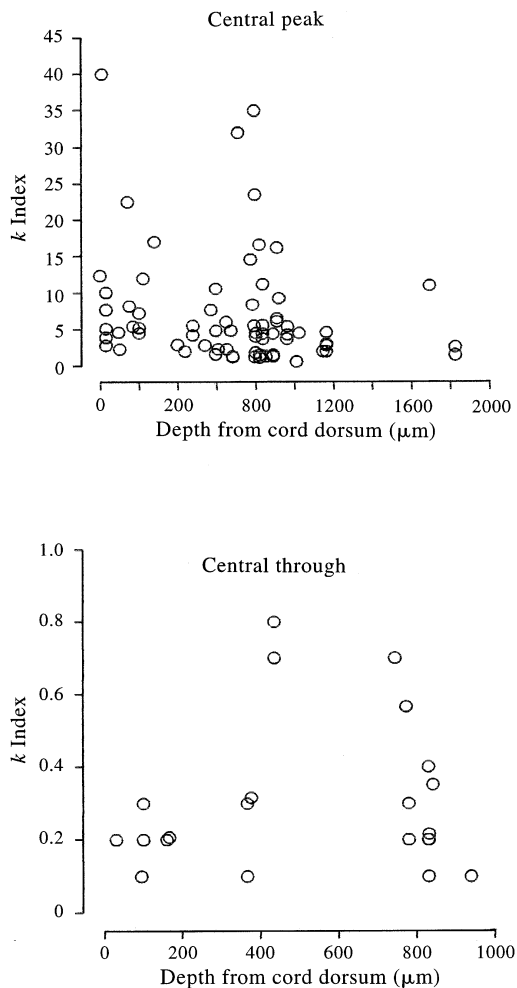
Fig. 3. Strength of neuronal interaction measured by the *k* index vs depth of recording site for cross-correlograms suggestive of common input i.e. with a central peak or a central trough. Differences between both ordinates are due to differences in *k* index for peaks or troughs.

(Increase pattern, Fig. 4B) which was found in 62 (27.9%) neurons distributed over all laminae as well (Fig. 4D). The relationship between these discharge patterns and those of the cross-correlation where these neurons were involved is presented in Table 4. Peak autocorrelation was the most frequent pattern (84.1%) among neurons which present cross-correlations with central peak patterns. On the other hand, cross-correlation with central trough or with delayed peak were associated significantly with the increase autocorrelation pattern in 76.3% and 83.4% of the neurons, respectively. The incidence of the two types of autocorrelation patterns was the same for flat cross-correlation.

Rhythmic discharges and cross-correlation patterns

Cross-correlation of neuron pairs in which one or both or no cells displayed rhythmic discharges was evaluated. Patterns with bilateral peaks presented the highest incidence of rhythmic cells (9/14, 64.3%, $p<0.01$) from the other cross-correlation patterns (Table 5). Pairs of rhythmic neurons which displayed bilateral peaks in their cross-correlation always presented different fundamental frequencies. The time-lag between the peaks in the autocorrelation did not correspond to the time-lag of bilateral peaks in the cross-correlation of these neurons.

Effects of long-lasting skin heating on the cross-correlation patterns

The cross-correlation patterns of 37 pairs of neurons were evaluated for background activity and also during long-lasting skin heating (48°C, 100 s) at the receptive field of both cells when this was possible, or at one of them and close to the other. Individual neuronal responses were similar to those described previously by Cervero et al.,[8] for prolonged noxious mechanical stimulation, e.g., fast strong increase of the discharge rate followed by tonic discharge at a higher level than the control toward the end of the stimulation. During the stationary tonic phase, heat stimulation induced qualitative changes in the cross-correlation patterns of 8/37 (21.6%) pairs of neurons (Table 6, Fig. 5). Of these, four pairs changed to non-correlated discharges (flat cross-correlation) and the remaining four pairs changed to other patterns. Skin heating induced no qualitative change in 29/37 (78.4%) cross-correlations (Table 6), but quantitative changes (*k* and/or width values) were observed in 18/21 (85.7%) neuronal pairs.

Effects of radiant heat-induced skin inflammation

Twenty-six pairs of neurons were recorded with intact skin and then up to 220 min after the induction of inflammation by radiant heat at the receptive field or close to the receptive fields. Eight pairs (30.8%) of neurons qualitatively changed their cross-correlation patterns during skin inflammation. Three of these changed their patterns from central peak to bilateral peaks. An example of this change is shown in Fig. 6. Changes were observed at the first recording (60 min) after the beginning of the induction of inflammation; these changes reversed to control 220 min thereafter. In one neuron pair the changes were not reversible up to 220 min after the induction of inflammation. Increases in the area of mechanoreceptive fields in 23 of 34 (67.6%) fully-tested neurons were observed during inflammation of the skin. In 4/34 (11.8%) neurons a decrease was observed, and 7/34 (20.6%) neurons could not be fully tested. Inflammation-induced changes in the receptive fields were evaluated in 13 pairs of neurons. With the skin intact, 7/13 (53.8%) neuron pairs had completely overlapping receptive fields; this figure increased to 11/13 (84.6%) with the skin inflamed.

Table 2. Cross-correlation patterns of background discharges from pairs of functionally-classified spinal dorsal horn neurons

| Neuron pair | Cross-correlation patterns | | | |
	Central peak	Central trough	Bilateral peak	Flat
Two LT	5/12 (41.7)	4/12 (33.3)	1/12 (8.3)	2/12 (16.7)
LT-MR	8/14 (57.1)	3/14 (21.4)	2/14 (14.3)	1/14 (7.2)
Two MR	41/65 (63.1)	9/65 (13.8)	3/65 (4.6)	12/65 (18.5)

Incidence is reported as number of found neurons/number tested, percentage is presented in parentheses. Statistical significance was tested using the z-test. No significant difference was found among incidence groups. LT, low threshold neuron; MR, multireceptive neuron.

Table 3. Functional synchronization and overlap of cutaneous mechanoreceptive fields of simultaneously recorded dorsal horn neurons with the cord intact.

Cross-correlation pattern	Overlapping	Non overlapping	Total
Central peak	45 (91.8)	4 (8.2)	49
Central trough	16 (100)	— —	16
Bilateral peak	4 (57.1)	3 (42.9)	7
Flat	7 (63.6)	4 (36.4)	11

Number of neuron pairs analysed for each correlogram pattern is given (in parenthesis). Incidence was calculated as number of found neuronal pairs / total number of neuronal pairs with the same pattern.

Effects of spinalization and anaesthesia on auto- and cross-correlation patterns

Changes in the cross-correlation patterns induced by reversible cold block spinalization at the cervicothoracic cord were evaluated in 36 pairs of neurons. Six (16.7%) pairs presented qualitative changes in their cross-correlation patterns during spinalization (Fig. 7, Tables 7 and 8). From these six, only one changed from a correlated pattern (central peak) to an uncorrelated one (flat). In decerebrate, unanaesthetized, spinalized rats, autocorrelations with peak pattern were observed in 18/20 (90%) neurons, the remaining two neurons presented increased autocorrelation pattern. For cross-correlograms, central peak patterns were observed in 7/10 (70%) pairs of neurons, central trough was observed in one pair (10%), delayed peak in one pair (10%) and flat pattern in another pair (10%). These incidences were not statistically different from those observed in unlesioned, anaesthetized animals ($p > 0.05$).

Quantitative changes in firing synchronicity

Changes greater than 10% of control values in the synchronicity time (width of peak or trough in cross-correlation) or strength (k index) were analysed during three different experimental situations, i.e. long-lasting skin heating, cold block reversible spinalization and radiant heat-induced skin inflammation (Table 8). Long-lasting skin heating induced predominantly an increase of both k and time values. Skin inflammation induced decrease of synchronization time in about 60% of the cases, the other 40% did not changed. Incidence of changes in the k index during inflammation were not different between increase, decrease or no change. Decrease of synchronization time associated with similar incidence of increased or decreased synchronization strengths (50%) was observed during reversible spinalization.

DISCUSSION

In the present study adjacent spinal dorsal horn neurons were simultaneously recorded with a single electrode, and their discharges were analysed using point process methods. Functional connectivity as inferred from firing synchronicity, its modulation by supraspinal structures, the effect of tonic noxious stimulation of primary afferents and sensory processing by small networks were herein evaluated in the spinal dorsal horn. Neuronal network models inferred by means of cross-correlation of simultaneously recorded spike trains, a method based on the firing time synchronization, do not consider all possible states of all constituent neurons.[1] Hence in the present study the inferred functional connectivity should be considered as a minimal network model able to explain the synchronic discharge of the underlying neurons, i.e. common input and reverberating circuits.

Local, segmental and propriospinal dorsal horn networks

Evident signs of synchronization, i.e. cross-correlation patterns with central peak or central trough, were observed in the vast majority of neurons in all laminae. These patterns were not qualitatively changed in the majority of cases either by activation of nociceptive primary afferents or by spinalization.
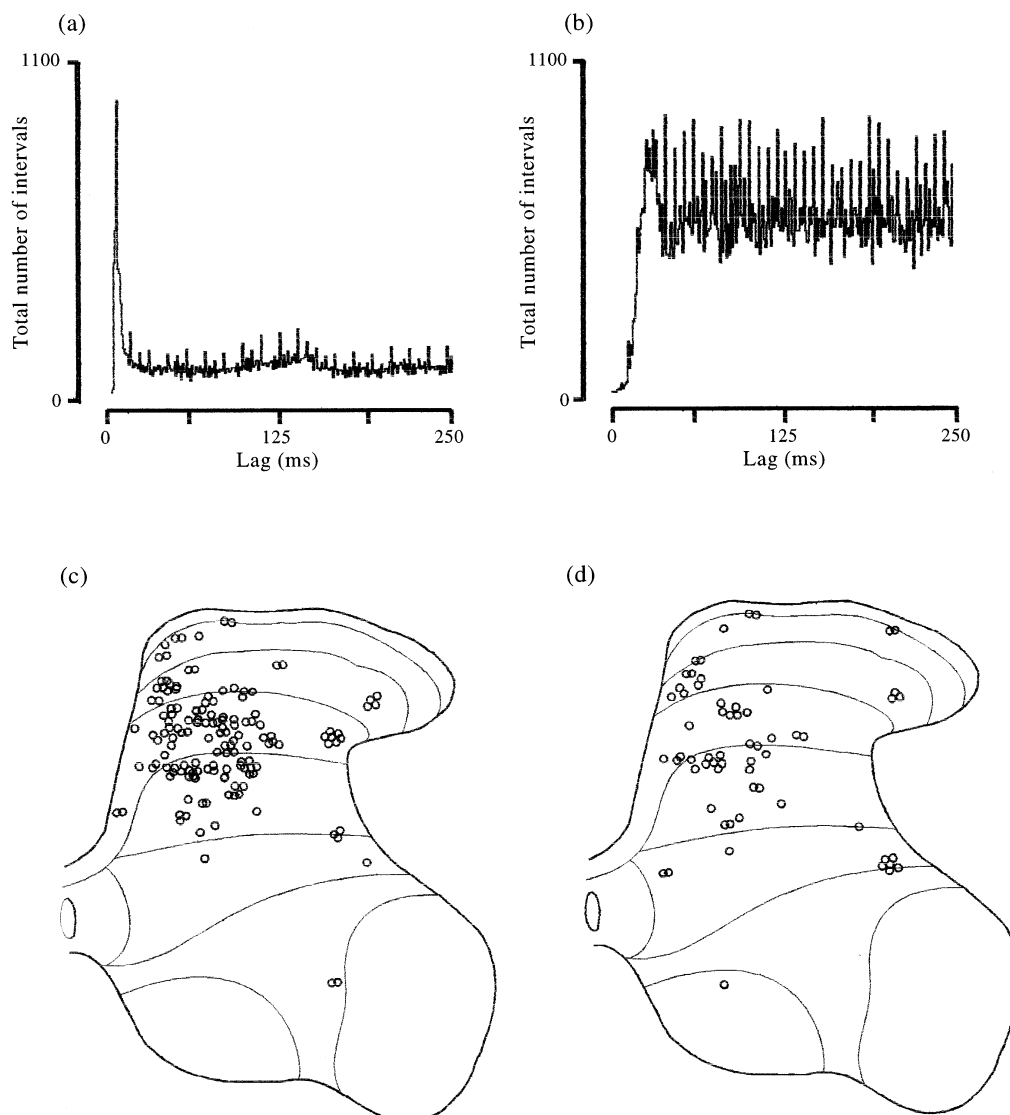
Fig. 4. Typical examples of autocorrelogram patterns of discharges from spinal dorsal horn neurons are presented. A) peak probability to fire shortly after the refractory period of an action potential (Peak pattern); B) reduced probability to discharge following an action potential with a slow recovery of the excitability (Increase pattern). The histologically verified recording sites where each autocorrelogram was recorded are presented in C and D, respectively, as superimpositions on a representative section through the lumbar spinal dorsal horn.

Primary afferent fibres, segmental neurons, propriospinal neurons and supraspinal structures could be postulated as sources of this synchronization. Anatomical data demonstrate that propriospinal neurons are by far the largest group of spinal neurons originating from and terminating in all laminae of the gray matter.[9] This provides the morphological substrate for a complex interaction between spinal neurons of different spinal segments. Functional evidences show that propriospinal neurons originating from well-defined areas of the thoracic, lumbar or sacral spinal cord control the activity of nociceptive neurons in the lumbar spinal cord.[55] However, the main sensory input to dorsal horn laminae III–VI originates from cutaneous mechanoreceptors with

no or rare background activity in the absence of mechanical skin stimulation.[65] These well documented descriptions of input from peripheral receptors suggest that they could not be the source of spinal background and synchronized activity. In contrast, the present study shows that pairs of neurons with correlated discharges showed the highest incidence of overlapping receptive fields and simultaneously the lowest incidence of non-overlapping ones. These results agree with those reported by Brown et al.,[6] for the cat. On the other hand, discharge synchronization associated with overlapping receptive fields does not necessarily mean that primary afferents are a significant source of synchronization. Overlapping receptive fields of multireceptive neurons in the cat are associated with an

Table 4. Incidence of autocorrelation and cross-correlation patterns from 223 spinal dorsal horn neurons

CC pattern	n	AC-p (%)	AC-i (%)
Central peak	132	84.1	15.9
Central trough	38	23.7	76.3
Bilateral peak	14	64.3	35.7
Delayed peak	12	50	50
Delayed trough	6	16.6	83.4
Central peak			
+ delayed trough	10	70	30
Flat	38	50	50

Incidence is reported as percentage of n from each pattern group. CC, cross-correlation; n, number of neurons which bellow to pairs with specific cross-correlation pattern; AC-p, autocorrelation with peak pattern; AC-i, autocorrelation with increase pattern.

Table 5. Cross-correlation patterns of background discharges of different combinations of rhythmic and non-rhythmic neurons, in spinal dorsal horn. Cross-correlation patterns

Neuron pair	Central peak		Central trough		Bilateral peaks		Flat	
Two R	19/29	(65.5)	3/29	(10.3)	3/29	(10.3)	4/29	(13.8)
R-NR	24/45	(53.3)	8/45	(17.8)	3/45	(6.7)	10/45	(22.2)
Two NR	26/42	(61.9)	10/42	(23.8)	1/42	(2.4)	5/42	(11.9)

Incidences are reported as number of found neurons/number tested, percentage is presented in parentheses. Pairs of rhythmic neurons with bilateral peaks in the cross-correlation of their discharges displayed in all cases (3) different discharge frequencies in their autospectra. Significance was calculated using the z-test. No significant difference was found among incidence groups. R, rhythmic neuron; NR, non-rhythmic neuron.

Table 6. Qualitative effect of long-lasting skin heating on cross-correlation patterns of spinal dorsal horn neuron pairs ($n=37$)

Response to stimulation	Cross-correlation patterns	Incidence	
No change:			
29 (78.4)	Central peak	24	(64.9)
	Central trough	4	(10.8)
	Bilateral peak	1	(2.7)
Changed:			
8 (21.6)	Central peak to flat	3	(8.1)
	Central trough to flat	1	(2.7)
	Bilateral to central peak	3	(8.1)
	Flat to central trough	1	(2.7)

Incidence is reported as number of found neuron pairs/number tested, percentage is presented in parentheses.

overlap of their dendritic trees.[5] Dendrodendritic synapses, which were postulated to be important in the generation of central peak patterns in cross-correlograms of medullary neurons,[16] might also be present in the spinal dorsal horn where dendrodendritic contacts have been described in the monkey[34] and the cat.[20] However this type of connectivity will induce a short-lasting central peak in cross-correlogram which was not seen, probably because it was masked by other long-lasting interactions evidenced by broad central peaks which may indicate that activation of recorded cells occurs with varying latency and order, possibly due to the existence of parallel and multisynaptic cascading interneuronal pathways, and recently this interpretation has been extended to the possibility of a reciprocal neuronal interaction.[35] Electrotonic coupling, another theoretically possible source of synchronicity, has not been described in spinal dorsal horn.

Discharge synchronicity as epiphenomenon of the system's properties should also be considered;[59] the fact that neurons with correlated burst discharges may display cross-correlation patterns of common input has been described by Gochin *et al.*,[21] for neurons in the inferior temporal cortex. In the present study all other cross-correlation patterns have the same incidence of burst neurons, suggesting that in our neuronal sample burst activity is not determinant for cross-correlation patterns.

The strength of interaction for neurons with common input was not statistically different between superficial and deep laminae, suggesting that the
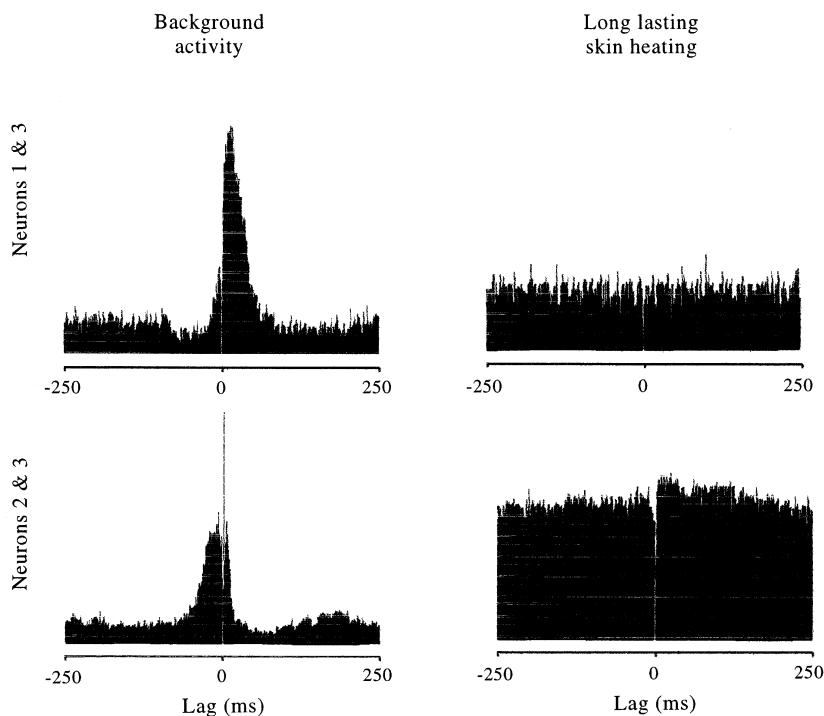
Fig. 5. Cross-correlograms of background discharges and of discharges at the stationary phase of long-lasting skin heating (48°C, 100 s), from three simultaneously-recorded spinal dorsal horn neurons. Note that during background activity both neuronal pairs presented common input patterns expressed by a central peak, which was completely abolished during skin heating. Ordinates of histograms are normalized only for illustration purposes.

synchronization strength is exerted in a similar form for both neuronal groups.

Our results show that despite the well known mediolateral somatotopic and laminar organization of the dorsal horn,[66] the sensory network of adjacent neurons is not constituted by clusters of neurons with similar function, i.e. multireceptive, low threshold or nociceptive specific, but by a mixed group of them.[53]

It is well known that reverberating neuronal circuits are expressed in the cross-correlation as bilateral peaks,[42] and the present study has shown very similar patterns in the spinal dorsal horn. The incidence of cross-correlation patterns with bilateral peaks was larger for neuron pairs when one or both cells were rhythmic. This type of pattern may arise as an epiphenomenon if both neurons discharge with the same frequency.[47] This possibility was excluded in the present study by the fact that in all these pairs of neurons no similar or harmonic frequencies were observed. Furthermore, bilateral peaks in the cross-correlogram were found in pairs of rhythmic or non-rhythmic neurons located only in laminae IV and V. Neurons in these laminae frequently present prominent dorsal dendrites that stretch into superficial laminae and form extended dendritic trees (see Ref. 65 for a review), as well as axonal arborizations in the region of the dendritic tree.[41] These morphological arguments suggest the existence of reciprocal connections between these cells which are able to generate cyclic excitation expressed as bilateral peaks in the cross-correlation. Common input with opposite polarity or reciprocal inhibition, have been proposed as rhythmogenic factors,[40,52] but in the present study cross-correlation patterns suggestive of such a circuitry, i.e. central trough, were associated only with 33.3% of rhythmic discharging neurons. Other possibilities like feed-forward mechanisms are less likely to be present in this circuitry. More reciprocal excitatory interactions were expected to exist based on morphological evidence (see above). The recording volume of the microelectrode (average diameter 100 μm) includes pairs of neighbouring neurons located on the dorsoventral plain where more oligosynaptic interactions were expected. However, cross-correlograms with bilateral or delayed peaks or troughs were rare. It is possible that such patterns can be masked if the two neurons have additionally a long-lasting common input, i.e. a cross-correlogram with broad central peak. Thus, the incidence of cross-correlation patterns suggestive of reciprocal excitation is probably underestimated by using conventional cross-correlation method.[35] The existence of positive feedback circuitry in dorsal horn nociceptive neurons suggest the possible increase in their discharge rates and/or in discharge duration despite any additional input to the circuit, a phenomenon which can be involved in hyperalgesia and allodynia.
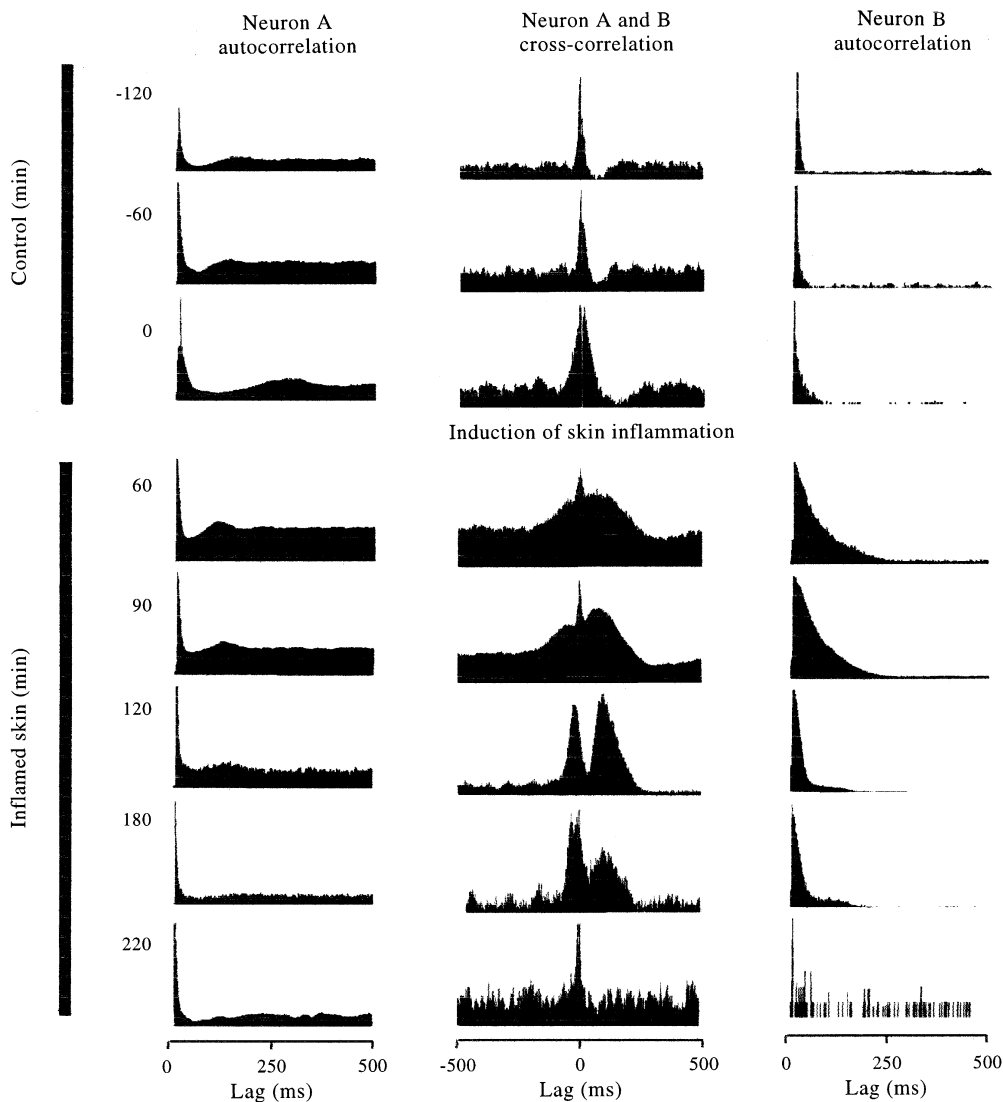
Fig. 6. Autocorrelation and cross-correlation of the discharges of a pair of spinal dorsal horn neurons before and during skin inflammation within the cutaneous mechanoreceptive field of one neuron and near the receptive field of the other. Each row of correlations was calculated from 15 min recording. Note in the controls a typical pattern of common input (central peak) to which an inhibitory contact from neuron A to B (delayed trough on the positive scale) is added. 90 min after the initiation of inflammation a bilateral peak takes place in the cross-correlation, and after 220 min the original pattern is restored. Autocorrelation of the discharges of each neuron reveals concomitant changes in the time-course of membrane excitability.

Peak autocorrelation was the most frequent pattern and was strongly associated with synchronized discharges, additionally, rhythmic discharging patterns were associated with bilateral peak cross-correlation patterns. These results support the notion, that the pattern of neuronal discharge may depend upon the properties of the functional connectivity at the recording site.

Influence of anesthesia on auto- and cross-correlation patterns

Autocorrelation patterns described in the present study have been found in other nociception-related areas of the CNS like ventromedial medulla[43] where autocorrelations with increase pattern were most frequent than peak pattern under barbituric anaesthesia. For our study, the opposite situation was observed i.e. peak pattern as most frequent pattern and rare incidence of increase pattern. These results suggest that for spinal dorsal horn neurons, barbituric anaesthesia is not associated with post-spike inhibition pattern (trough autocorrelogram) as was suggested for ventromedial medulla.[43] Despite that incidence of auto- and cross-correlation patterns obtained from unanaesthetized, decerebrated, spinalized rats were not statistically different from those obtained under barbituric anaesthesia, further studies of the level and type of anaesthesia are needed to elucidate its role.

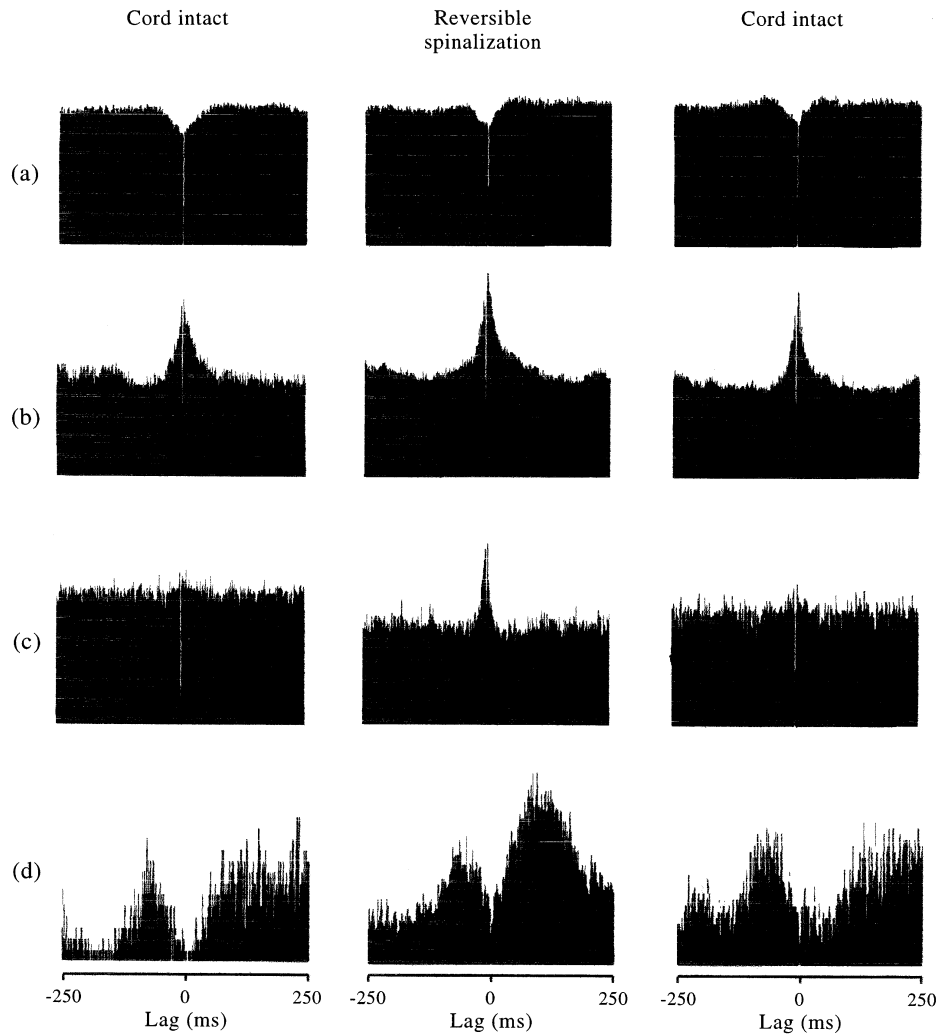Cord intact | Reversible spinalization | Cord intact

Fig. 7. Cross-correlograms of discharges from four different pairs (a–d) of simultaneously recorded spinal dorsal horn neurons, before, during and after reversible cold block spinalization at the cervico-thoracic junction. The neuron pair d was recorded during inflammation of the skin at the receptive fields of both neurons. Note that spinalization did not change the patterns of cross-correlation in a, b and d. Common excitatory input to both cells was reversibly induced during spinalization in c. Ordinates of histograms are normalized only for illustration purposes.

Table 7. Effects of spinalization on cross-correlation patterns of pairs ($n=36$) of spinal dorsal horn neurons

Response to spinalization		CC patterns	Incidence	
No change:		Central peak	19	(52.9)
	30 (83.3)	Central trough	3	(8.3
		Delayed peak	2	(5.5)
		Flat	6	(16.6)
Changed:				
	6 (16.6)			

Incidence is reported as number of found neurons pairs/number tested, percentage is presented in parentheses. CC, cross-correlation.

Stimulus induced plasticity of local spinal networks

The cross-correlation analysis of neuronal discharges is able to reveal changes in the functional connectivity among constituent neurons in a network.[2,12,19,31] Plasticity, understood as changes in the local functional synchronicity induced by primary afferent activation were evaluated during two

Table 8. Quantitative changes induced by long-lasting skin heating, cold block reversible spinalization or radiant heat-induced skin inflammation in the synchronization time (Peak or trough width) or strength (k index) of those cross-correlogram patterns suggestive of common input (central peak and central trough) of simultaneously recorded dorsal horn neurons

		Time		Strength	
Heat	Increase	27	(73)	23	(62.2)
$n=37$	Decrease	5	(13.5)	12	(32.4)
	No change	5	(13.5)	2	(5.4)
Inflam	Increase	—		11	(42.3)
$n=26$	Decrease	15	(57.7)	9	(34.6)
	No change	11	(42.3)	6	(23.1)
Spin	Increase	10	(27.8)	18	(50)
$n=36$	Decrease	23	(63.9)	18	(50)
	No change	3	(8.3)	—	

Incidence is reported as number of found neurons pairs, percentage related to experimental group is presented in parentheses. Changes greater than 10% of control values were used as cutoff criterion. Heat, long-lasting skin heating; Spin, cold block reversible spinalization; Inflam, radiant heat-induced skin inflammation.

different experimental conditions, namely long-lasting radiant skin heating and heat-induced skin inflammation. Qualitative changes of functional synchronicity were observed in about 20% of the neuron pairs. These changes probably did not reflect modifications in the morphological array of the network, e.g., new synapses, because they occurred and disappeared in a short period of time. They are probably the expression of activation, inhibition and/or modulation of pre-existing synaptic contacts as a response to the release of neuromodulators within the neuronal network (cf. Harris-Warrick and Marder[24]) or under the main concept of volume transmission, i.e. non-synaptic, ephatic, paracrine and/or neuroendocrine transmission.[18,54,62] On the other hand, controlled superfusion of the spinal dorsal horn with substance P failed to qualitatively change the patterns of cross-correlation but was able to change neuronal discharge patterns.[39] Quantitative changes in the functional synchronicity, i.e. changes in the strength of synchronicity, were observed in the present study as responses to primary afferent activation. Long-lasting radiant skin heating induced in the vast majority of neuron pairs an increase of the synchronization. The differential quantitative changes expressed by the local functional network as response to long-lasting skin heating or radiant heat-induced skin inflammation are evidence of the plastic capabilities based mainly on differential changes of the strength and/or the synchronization time. The changes discussed above support the notion of stimulus-induced plasticity, and can be associated with sensitization of spinal sensory neurons. The existence of reciprocal excitation among multireceptive and/or low-threshold dorsal horn neurons suggests the possibility that during pathophysiological conditions such as hyperalgesia, this functional interaction may play an important role.

The present study shows the existence of a functional synchronicity in the spinal dorsal horn that can be modified mainly quantitatively by activation of nociceptive primary afferents. These changes might imply the opening of a functional pathway interpreted as a new network condition with specific processing function for this information. The results show that this stimulus-induced plasticity occurred in about 60% of the cases by modulation of strength i.e. quantitative changes in the synchronicity, and in about 20–23% of the cases by actual new functional ''wiring'' of the network. Such stability in the qualitative ''wiring'' provides the network with the necessary reference framework to perform its original task. On the other hand, a rigid network without some plasticity would be unable to assume new dynamic ranges or modes of operation.[24] Noxious long-lasting skin heating induced an increase of both the strength and the time of synchronization, suggesting a neuronal recruitment process which may be based on temporal and spatial summation and not on the general asynchronic increase in activity. On the other hand, skin inflammation induced more complex changes, i.e. decrease (better timing among discharges from different neurons) or no changes in synchronization time, or no change in the synchronization time and nearly similar incidence of increase, decrease or no change in the synchronization strength. This complexity might indicate that, within the recording time, the inflammation-induced changes were in evolution as shown in Fig. 7. More interesting are the qualitative changes where the activation of excitatory reverberate circuitry during skin inflammation was observed. These possibilities should be considered as additional mechanisms to explain pathophysiological conditions such as hyperalgesia and allodynia, and need to be further investigated.

Influence of tonic descending systems to spinal dorsal horn neuron synchronicity

The results of reversible spinalization show that the tonic descending systems to the spinal dorsal horn have a partial role in the maintenance of functional synchronicity of local neuronal networks. The results reported here give evidence that the supraspinal structures exert a modulation on the functional synchronicity of dorsal horn neurons by a prolongation of the synchronization time. This suggests that supraspinal structures may act through a complex polysynaptic chain toward the dorsal horn. Differential effects on the synchronization strength were always observed with similar incidence for increased or decreased values of k, supporting the notion of the existence of multiple, parallel and possibly independent descending modulatory systems to the dorsal horn neurons. This agrees with experimental data from Sandkühler et al.,[54] who found differential effects of spinalization on discharge rates and discharge patterns of neighbouring dorsal horn neurons. The qualitative changes of cross-correlation patterns observed during spinalization in about 17% of neuron pairs suggest that, although not essential, supraspinal modulation can contribute to maintaining local functional synchronicity. The modulation of dorsal horn neurons by tonic descending systems is expressed by a reduction of the synchronization time which may be associated with increase or decrease in the synchronization strength. These facts also agree with reports of differential effects of spinalization on the background activity of simultaneously-recorded dorsal horn neurons (see above), supporting the notion of multiple parallel descending pathways with different final effects.

Sensory processing

The vast majority of adjacent neurons shared many common inputs as revealed by the high incidence of cross-correlograms with central peak or central trough, but this probably does not occur for more separated neurons. Indeed, Brown et al.,[6] reported for the cat only 12% of positive cross-correlations between lumbar dorsal horn neurons with pattern of synchronization which were recorded with two micropipettes 1000–1500 μm apart. These results taken together show a distance-dependent drastic reduction in functional synchronicity.

The co-operative activity of a spatially-distributed neuronal subpopulation could constitute a cell assembly which encodes a stimulus by means of correlated increases of neuronal discharge rates.[1] Such a processing scheme has been proposed for the visual cortex[15,36,56] and olfactory information processing.[30] Additionally, it was suggested that temporal firing synchronization rather than uncoupled individual activity increases is important for the stimulus encoding by the neuronal assembly. The high incidence of firing synchronicity of dorsal horn neurons reported in the present study agrees with the notion that these neurons are organized in functional assemblies. A neuronal assembly with multiple inputs (multireceptive, low threshold and nociceptive specific) which may use different stimulus coding strategies depending on the different functional connectivities of the neural network. In this sense, plasticity constitutes not only a consequence of long-lasting noxious input but also a new processing scheme for this input afforded by the fact that constituting neurons of the assembly may change their functional intrinsic properties.[63] Further and different approaches are needed to elucidate whether these synchronized cells use a redundant, independent or synergistic processing strategy for sensory inputs.

CONCLUSIONS

The present study provides evidence that the local spinal dorsal horn network is mainly formed by a mixed group of neurons, i.e. multireceptive, nociceptive specific and low-threshold neurons, rhythmic and non-rhythmic neurons and that discharge synchronization is the main characteristic of the network. This synchronization is very stable during background activity. Signs of oligosynaptic interaction were rare but its existence may be masked by the strong expression of the propriospinal input. Cutaneous, propriospinal or supraspinal sources of input to local network excert their effects mainly on the strength and/or time of the neuronal synchronization and less by activation of new neuronal connectivity. The presence of reverberating circuitry located in deep laminae and its role in physiopathological conditions like hypersensibilization should be studied further.

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