Temperature sensitivity of neurones in slices of the rat spinal cord

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1. The inherent temperature sensitivity of 343 spontaneously active neurones recorded from rat spinal cord (SC) slices was investigated electrophysiologically. Recordings were made from 321 neurons from transverse and 22 neurons from longitudinal slices and their thermosensitivity was determined by relating changes in firing rate to changes in slice temperature.

2. Of the neurones from transverse slices, 53% were warm sensitive, 2% were cold sensitive and 45% were temperature insensitive. In longitudinal slices, 68% were warm sensitive and the remaining neurones were temperature insensitive.

3. When classified according to their recording sites in transverse slices, warm-sensitive neurones in laminae I and II had the same mean temperature coefficient compared with those recorded from lamina X, despite the fact that the latter had a significantly higher spontaneous activity.

4. The intrinsic temperature sensitivity of the majority of warm-sensitive neurones was confirmed by blocking their synaptic input.

5. A transient overshoot in activity, i.e. a dynamic response characteristic following rapid temperature stimuli (0·4 °C s^{-1}) was observed in 73% of the warm-sensitive and 59% of the temperature-insensitive neurones in laminae I and II in response to rapid warming, but only rarely (< 10%) in lamina X.

6. Temperature-sensitive SC neurones share response characteristics with temperature-sensitive neurones in the preoptic and anterior hypothalamic (PO/AH) area and with peripheral temperature receptors. Functionally, these neurones may represent the cellular basis for the temperature sensory function of the spinal cord that has been well characterized *in vivo* in homeothermic species.

The importance of the spinal cord (SC) for the regulation of body temperature has, like that of the preoptic area and the anterior hypothalamus (PO/AH), been demonstrated in various homeothermic species (Simon, 1974; Necker, 1975), including the rat (Lin, Yin & Chai, 1972). These *in vivo* studies have shown that local warming of the SC elicits responses contributing to heat loss (panting, sweating and vasodilatation), whereas local cooling of the SC elicits responses contributing to heat gain (shivering, vasoconstriction, piloerection, decreased respiratory rate and increased food intake). When the lumbar SC is warmed or cooled, the corresponding thermoregulatory responses such as panting (Jessen & Simon-Oppermann, 1976) or shivering (Meurer, Jessen & Iriki, 1967) persist even after dorsal root transection, indicating that primary thermosensory elements whose signals drive these responses are located within the spinal cord.

Unlike the cold-induced shivering, which might be due in part to the local cold sensitivity of spinal motoneurones (Pierau, Klee & Klussmann, 1969, 1976), the panting induced by local warming of the lumbosacral SC must be due to an ascending propagation of the signals from temperature sensors within the SC. These temperature-sensitive sensory afferent neurones were subsequently characterized by recording single unit activity in the ascending anterolateral tract of the cervical SC (C2–C4) after local temperature stimulation of the lumbar SC of cats (Simon & Iriki, 1970, 1971*a*, *b*) and pigeons (Necker, 1975).

Attempts to evaluate the neuronal properties underlying the thermosensory function of certain nervous regions have so far been concentrated on the PO/AH, and during the past decade the thermosensitivity of neurones in this area has been characterized largely by studies of tissue slices

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(Boulant & Dean, 1986; Boulant, 1994). Various criteria have been proposed for classifying these neurones according to the temperature coefficient (TC) of their firing rates (FR) in response to temperature changes and it has been shown for neurones of the PO/AH that their warm sensitivity and rarely also the cold sensitivity was inherent and not the result of synaptic networks (Hori, Nakashima, Kiyohara, Shibata & Hori, 1980; Kelso & Boulant, 1982). Spinal cord slices offer an experimental advantage over hypothalamic tissue slices because SC neurones in afferent paths are anatomically separated from SC neurones in efferent paths, since afferent signals are transmitted to the dorsal horn and the efferent signals are transmitted from the ventral horn.

The aim of this study was to characterize electrophysiologically the inherent temperature responsiveness of spontaneously active neurones recorded from SC slices so as to permit comparison with thermosensitive neurones in PO/AH slices. To investigate the possible influence of intersegmental synaptic input on the properties of temperature-sensitive neurones, the responses of neurones recorded from longitudinal and transverse SC slices were compared. The thermosensitivity of neurones was also evaluated after synaptic interaction had been blocked.

The recordings from transverse slices were restricted to the outer layers of the dorsal horn (laminae I and II) and the area around the central canal (lamina X), because histological and electrophysiological evidence has suggested that these two areas are the most likely sites of spinal thermosensitivity. The number of c-fos-positive neurones in the spinal cord of rats exposed to cold ambient temperatures increased significantly primarily in laminae I and II and lamina X (Kiyohara, Miyata, Nakamura, Shido, Nakashima & Shibata, 1995). A similar but less obvious pattern of neuronal c-fos activation has been seen in warm-exposed animals. The results of this histological study, along with the electrophysiological evidence that every local temperature-sensitive SC neurone receives input from peripheral temperature receptors (Simon, 1972), suggest that laminae I and II and lamina X are the spinal areas most likely to contain temperature-sensitive neurones.

In this first characterization of thermosensitive neurones from SC slices, ramp-like as well as sinusoidal temperature changes between 33 and 41 °C were applied and neurones were classified using criteria the same as or similar to those used to classify neurones in the PO/AH (Hori, Nakashima, Kiyohara & Shibata, 1982; Boulant & Dean, 1986; Schmid & Pierau, 1993).

Dynamic temperature responses have been observed *in vivo* in about 50% of the ascending fibres from temperature-sensitive units (Simon & Iriki, 1971*b*). To detect and characterize dynamic responses of individual SC neurones, this study also used fast, step-like temperature changes. Dynamic responses to rapid temperature changes are one of the major criteria used to identify cutaneous thermoreceptors (Hensel, 1981) and might also be characteristic of CNS thermoreceptors.

METHODS

Experiments were carried out on slices of the spinal cord of adult male Wistar rats (150–320 g) which had been anaesthetized with 1·5 mg (kg body wt)$^{-1}$ urethane (Sigma) I.P. before laminectomy and decapitation in initial experiments ($n = 26$). In subsequent experiments ($n = 159$), anaesthesia was omitted and rats were decapitated with a guillotine specifically designed for rats. The upper lumbar spinal cord region was exposed and quickly superfused with ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (mM): NaCl, 124; KCl, 5; NaH_2PO_4, 1·2; $MgSO_4$, 1·3; $CaCl_2$, 1·2; $NaHCO_3$, 26; glucose, 10; equilibrated with 95% O_2 and 5% CO_2; pH 7·4; 290 mosmol kg^{-1}.

After cutting the dorsal and ventral roots, a section (10 mm) of the spinal cord comprising the segments L2–L4 was quickly removed and transferred into ice-cold ACSF for about 1 min. Two or three longitudinal or five to ten transverse slices (500 μm thick) were cut with a custom-made tissue slicer and preincubated at 35 °C for at least 1 h, before the first slice was transferred to the recording chamber and fixed to the bottom with a small platinum weight.

The gold-plated recording chamber was made from solid brass and contained a fluid volume of about 0·7 ml. The temperature was kept constant at 37 °C by means of a Peltier element. The chamber was constantly perfused with ACSF at a rate of 1·6 ml min^{-1}. ACSF entering the recording chamber was prewarmed to the same temperature as the solution already present in the chamber. Extracellular recordings were made with glass-coated platinum–iridium (Pt–Ir) electrodes from various layers of the spinal cord. In transverse slices spontaneously active neurones from the superficial dorsal horn (laminae I and II) and around the central canal (lamina X) were investigated for their local temperature sensitivity. The two regions were easily identified by the different visual appearance of the substantia gelatinosa comprising laminae I and II and lamina X, which is the area around the central canal. Recordings from longitudinal slices were made exclusively from the dorsal horn, but the exact recording site was not determined further. After a stable recording of a single neurone had been established, the temperature sensitivity of this neurone was determined by changing the temperature between 33 and 41 °C, either ramp-like with a velocity of 0·02 °C s^{-1} or sinusoidally, each procedure lasting 7 min. Fast, step-like temperature changes (0·4 °C s^{-1}, steps of 2 °C) were also performed in order to detect possible dynamic responses. The recorded action potentials were amplified and displayed on a storage oscilloscope and, after passing a window discriminator, they were fed into a personal computer via an interface and analysed using Spike2 software (both from Cambridge Electronic Design, Cambridge, UK).

The spontaneous activity of each neurone was evaluated by averaging its activity for 60 s prior to the first temperature stimulus.

The slope of the regression line, which resulted from plotting the FR of a neurone against the sinusoidally or ramp-like changes in temperature, determined the TC of a cell using a bin width of 10 s. A statistical program, which fits either one or two regression lines (Vieth, 1989) to a set of data, calculated the point of inflection

between temperature ranges exhibiting different TCs, without requiring any further assumptions. Cells were classified as being warm or cold sensitive when either the one (linear regression) or the steeper of the two regression lines (piecewise regression) had a slope (i.e. TC) exceeding +0·8 or −0·6 imp s^{-1} $°C^{-1}$, respectively.

For the warm-sensitive neurones recorded from transverse slices the sensitivity in terms of Q_{10} was calculated from the temperature (T) and the respective discharge rate (FR) for each regression line (linear or the steeper of the two regression lines) from the co-ordinates of its lowest (T_1, FR_1) and highest (T_2, FR_2) frequency by applying the Arrhenius formula,

$$\frac{FR_2}{FR_1} = e - \frac{\mu}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right),$$

where μ is the critical incremental energy of activation and R is the gas constant, and from the definition of Q_{10},

$$Q_{10} = e - \frac{\mu}{R}\left(\frac{1}{T+10} - \frac{1}{T}\right),$$

where $T = 310$ K. As a minimum discharge rate, FR = 1 Hz was used in order to prevent overestimation of the sensitivity of neurones with a low FR.

No differences were observed in the number and properties of warm- and cold-sensitive and temperature-insensitive neurones in transverse slices of the lumbar spinal cord from those rats that were anaesthetized prior to the decapitation (warm sensitive 51%, cold sensitive 4%, temperature insensitive 45%) and from the rats directly decapitated before the preparation of the tissue slices (53, 2 and 45%, respectively). Furthermore, there were neither differences between the two groups in the mean spontaneous FR at 37 °C nor in the mean temperature coefficient (TC) of warm-sensitive neurones (FR 12·3 ± 2·5 *vs.* 10·4 ± 0·7 imp s^{-1} and TC 1·9 ± 0·3 *vs.* 1·8 ± 0·1 imp s^{-1} $°C^{-1}$). Similarly, no differences were observed for the cold-sensitive neurones.

In order to block the synaptic input, slices were superfused with ACSF with a reduced Ca^{2+} (0·3 mM) and elevated Mg^{2+} (9·0 mM) concentration for at least 10 min before their temperature sensitivity was determined again.

If the transient increase or decrease in activity in response to a step-like temperature change exceeded ±30% of the corresponding tonic activity, the neurone was considered as being dynamic.

Average values in the text and in the tables are presented as means ± standard errors of the mean (S.E.M.). Statistical tests were performed on a computer using SigmaStat software (Jandel Scientific, Erkrath, Germany). Differences in spike amplitude, FR, TC and Q_{10} between various subsets of neurones were analysed using the Mann–Whitney rank sum test. The Friedman repeated measures analysis of variance on ranks, including multiple comparisons with the Student–Newman–Keuls *post hoc* test, was used to detect significant differences in the spike amplitudes at different temperatures of individual neurones and in the responses (TC) to different temperature stimuli of identical neurones. The Wilcoxon signed rank test was used when the responses to only two different stimuli were compared. To compare the numbers of neurones that fall into different categories, the χ^2 test was used. Differences are described as significant at a P value $< 0·05$ and as highly significant at $P < 0·01$.

RESULTS

Recordings from longitudinal and transverse SC slices

Signals from a total of 343 neurones were recorded in tissue slices from the lumbar spinal cord of 185 rats. Of these neurones, 321 were found in transverse slices, and 22 in longitudinal slices. At 37 °C the basal activity of the neurones in the transverse slices (7·6 ± 0·4 imp s^{-1}) did not differ significantly from that of the neurones in longitudinal slices (9·6 ± 2·6 imp s^{-1}). Of the neurones in transverse slices, 53% were warm sensitive, 2% were cold sensitive, and 45% were temperature insensitive. In the longitudinal slices, 68% were warm sensitive and the remainder were temperature insensitive. The comparison of the mean FR at 37 °C and the mean TC of the warm-sensitive neurones revealed neither a difference in the FR (10·7 ± 0·7 imp s^{-1} in transverse slices *vs.* 13·5 ± 3·4 imp s^{-1} in longitudinal slices) nor in the TC (1·8 ± 0·1 imp s^{-1} $°C^{-1}$ in transverse slices *vs.* 1·5 ± 0·2 imp s^{-1} $°C^{-1}$ in longitudinal slices). The mean FR of the cold-sensitive neurones in transverse slices was 5·9 ± 2·6 imp s^{-1}, and their mean TC was −1·6 ± 0·3 imp s^{-1} $°C^{-1}$. In both kinds of slices the mean FR of warm-sensitive neurones was significantly higher than that of temperature-insensitive neurones.

Temperature dependence of spike amplitude

The spontaneous action potentials of the neurones recorded from transverse slices had a mean amplitude of 250 ± 15 μV, yielding an average signal-to-noise ratio of 12·5 : 1 with a background noise of ⩽ 20 μV (Fig. 1). The mean amplitude of the spontaneous action potentials was the same

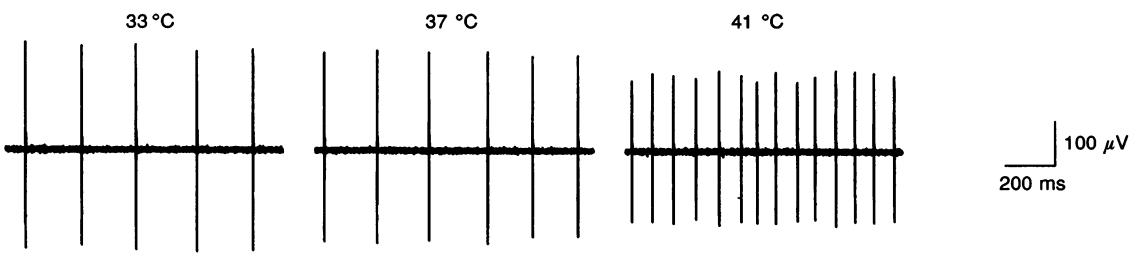

Figure 1. Effect of temperature on the spike amplitudes of a warm-sensitive neurone

The action potentials were recorded during a temperature stimulus at 33, 37 and 41 °C. The amplitude of the action potentials of this warm-sensitive neurone reversibly decreased with higher temperatures.

regardless of whether or not the neurones were temperature sensitive, but significant changes of the spike amplitudes of individual neurones could be observed at different temperatures (Fig. 1). For warm-sensitive neurones ($n = 51$), the spike amplitude at 41 °C (178 ± 24 μV) was significantly smaller than that at 37 °C (203 ± 28 μV) and the spike amplitude at 33 °C (223 ± 31 μV) was significantly larger than that at 37 °C. For temperature-insensitive neurones ($n = 38$), the spike amplitude was also smaller at 41 °C (259 ± 33 μV) than at 37 °C (272 ± 34 μV), but at 33 °C no difference could be observed (280 ± 36 μV).

Properties of temperature-sensitive neurones

The frequency and properties of warm-sensitive, cold-sensitive and temperature-insensitive neurones recorded from laminae I and II and lamina X of transverse slices are summarized in Table 1. Typical recordings of warm-sensitive neurones in these areas are illustrated in Fig. 2, and an example of a cold-sensitive neurone in lamina X is shown in Fig. 3. In laminae I and II the mean FR was significantly lower (4·2 ± 0·3 imp s^{-1}) than in lamina X (11·4 ± 0·8 imp s^{-1}). Classified according to their temperature sensitivity, the warm-sensitive neurones in laminae I and II had a significantly lower FR (5·5 ± 0·4 imp s^{-1}) than those recorded in lamina X (15·3 ± 1·0 imp s^{-1}), whereas there was no significant FR difference between the cold-sensitive neurones in laminae I and II (8·8 ± 5·4 imp s^{-1}) and lamina X (4·1 ± 2·7 imp s^{-1}), probably because so few cold-sensitive neurones were found. Despite the highly significant differences in the FR of warm-sensitive neurones, no differences in the TC could be observed between the warm-sensitive neurones in laminae I and II (1·6 ± 0·1 imp s^{-1} $°C^{-1}$) and those in lamina X (1·8 ± 0·1 imp s^{-1} $°C^{-1}$). The FR of the temperature-insensitive neurones in lamina X (6·2 ± 0·7 imp s^{-1}) was significantly higher than that of those in laminae I and II (3·0 ± 0·2 imp s^{-1}).

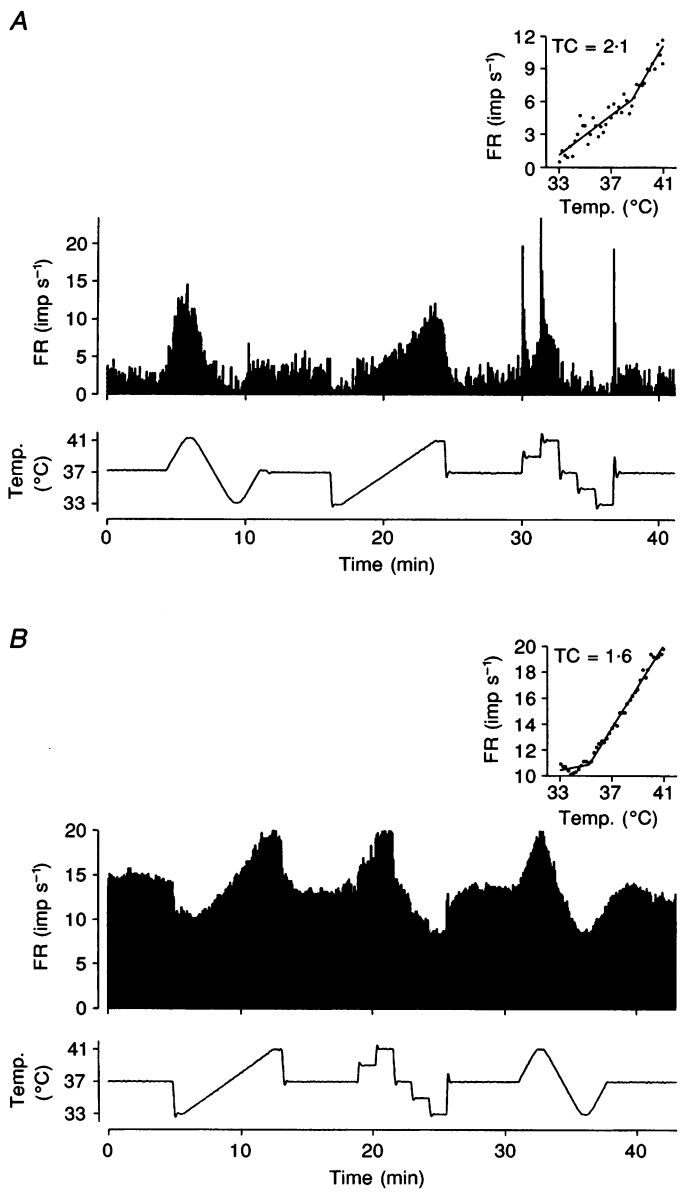

Figure 2. Continuous rate-meter recordings of firing rate (FR) and slice temperature (Temp) of two warm-sensitive neurones recorded from transverse SC slices

A, this neurone from lamina II had low basal activity and a tonic and dynamic temperature responsiveness. The temperature coefficient (TC) of this neurone was 2·1 imp s^{-1} $°C^{-1}$ for the upper temperature range. *B*, in contrast to the neurone in *A* this neurone from lamina X had a higher spontaneous FR and showed just tonic responses to various temperature stimuli (TC = 1·6 imp s^{-1} $°C^{-1}$ for the upper temperature range). The insets display the frequency–temperature relationships for the ramp-like temperature stimulus of each neurone.

Table 1. Basic properties of temperature-sensitive neurones recorded from transverse rat spinal cord slices

	Laminae I and II ($n = 183$)			Lamina X ($n = 116$)		
	Warm sensitive (46%)	Temperature insensitive (52%)	Cold sensitive (2%)	Warm sensitive (59%)	Temperature insensitive (37%)	Cold sensitive (4%)
Spontaneous FR* (imp s^{-1})	5·5 ± 0·4	3·0 ± 0·2	8·8 ± 5·5	15·3 ± 1·0	6·2 ± 0·7	4·1 ± 2·7
Temperature coefficient (imp s^{-1} $°C^{-1}$)	1·6 ± 0·1	0·3 ± 0·03	−2·3 ± 0·6	1·8 ± 0·1	0·3 ± 0·04	−1·1 ± 0·2
Irregular firing pattern † (%)	62	64	33	4	10	40
Dynamic temperature response ‡ (%)	73 (79)	59 (90)	0 (2)	9 (55)	3 (36)	50 (4)

* Average spontaneous FR at 37 °C; † amount of irregularly discharging neurones; ‡ percentage of neurones displaying a dynamic response to rapid temperature changes (number of neurones tested is given in parentheses).

When +0·6 imp s^{-1} $°C^{-1}$ is used as the criterion for warm sensitivity, as done previously when characterizing thermosensitive neurones in the PO/AH under identical conditions (Schmid & Pierau, 1993), 65% of all neurones in the present study were warm sensitive, 2% were cold sensitive, and the remaining 33% were temperature insensitive – and there were no differences between laminae I and II and lamina X in these percentages.

The temperature sensitivity of the warm-sensitive neurones in transverse slices was also calculated in terms of Q_{10}, in order to take the FR of the neurones into account. Since the calculated data were not normally distributed with a prevalence of high Q_{10} values, the geometric mean and the median of the data were used to describe the temperature sensitivity of the warm-sensitive neurones in terms of Q_{10}. In contrast to the mean TC, the mean Q_{10} value of the warm-sensitive neurones in laminae I and II was significantly higher (geometric mean, 9·3; median, 7·0) than that of the warm-sensitive neurones in lamina X (geometric mean, 3·8; median, 2·5).

Thermosensitivity of SC neurones after synaptic transmission was blocked

The temperature sensitivity of twenty-five spontaneously active SC neurones (13 warm sensitive, 1 cold sensitive, and 11 temperature insensitive) could be investigated further after their synaptic input was blocked by superfusing the slices with a solution that had a reduced [Ca^{2+}] and an elevated [Mg^{2+}]. Ten of the twenty-five neurones remained spontaneously active throughout the entire time of superfusion with the blocking solution (35–110 min). All the other cells ceased their spontaneous activity after 2–21 min in blocking solution. Nine of the thirteen warm-sensitive neurones retained their sensitivity in blocking solution, while the remaining four lost theirs. The TC of those neurones that retained their warm sensitivity was increased in four neurones and was reduced, but still above 0·8 imp s^{-1} $°C^{-1}$, in five neurones. Figure 4*A* shows an example of a warm-sensitive neurone that retained its temperature sensitivity in blocking solution. Although this neurone stopped its spontaneous activity after 9 min in

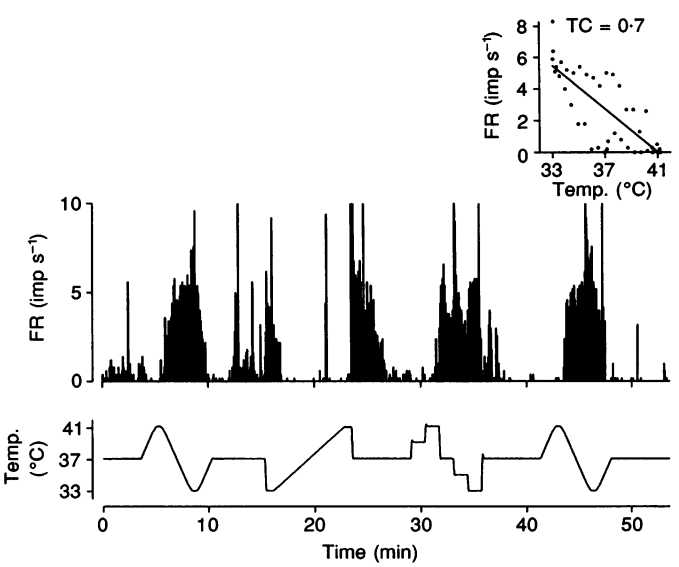

Figure 3. Cold-sensitive neurone recorded from lamina X of a transverse SC slice showing tonic and dynamic responses to various temperature stimuli

Inset, frequency–temperature relationship. The TC of this neurone was −0·7 imp s^{-1} $°C^{-1}$ and was linear over the entire temperature range tested.

blocking solution, its temperature sensitivity could be demonstrated clearly under these conditions. Its TC was even elevated (3·5 imp s^{-1} $°C^{-1}$) over that of the control (0·9 imp s^{-1} $°C^{-1}$), and the temperature–response curve was shifted to higher temperatures. The one cold-sensitive neurone (Fig. 4*B*) ceased its spontaneous firing after 2 min in blocking solution and no activity was recorded during the subsequent temperature change. Superfusing again with the normal perfusate restored the spontaneous activity and the temperature sensitivity of this neurone.

Two previously temperature-insensitive neurones were transformed into warm-sensitive neurones during superfusion with blocking solution, but lost this acquired thermosensitivity when the normal perfusate was restored. All the other temperature-insensitive neurones remained temperature insensitive. The neurone in Fig. 4*C* and the five other neurones tested with the blocking solution retained their dynamic temperature responses during synaptic blockade. Furthermore, the example in Fig. 4*C* shows that superfusion with blocking solution increased the TC of this previously temperature-insensitive neurone from 0·6 imp s^{-1} $°C^{-1}$ in the normal perfusate to 1·6 imp s^{-1} $°C^{-1}$.

Firing pattern of SC neurones in response to temperature changes

In lamina X, 89% of the neurones had a regular spontaneous firing pattern, whereas 63% of neurones from laminae I and II were irregular (see Table 1), a difference that was highly significant. Of the neurones in laminae I and II that were classified as irregular, 65% showed bursting activity. In lamina X, no bursting neurone could be

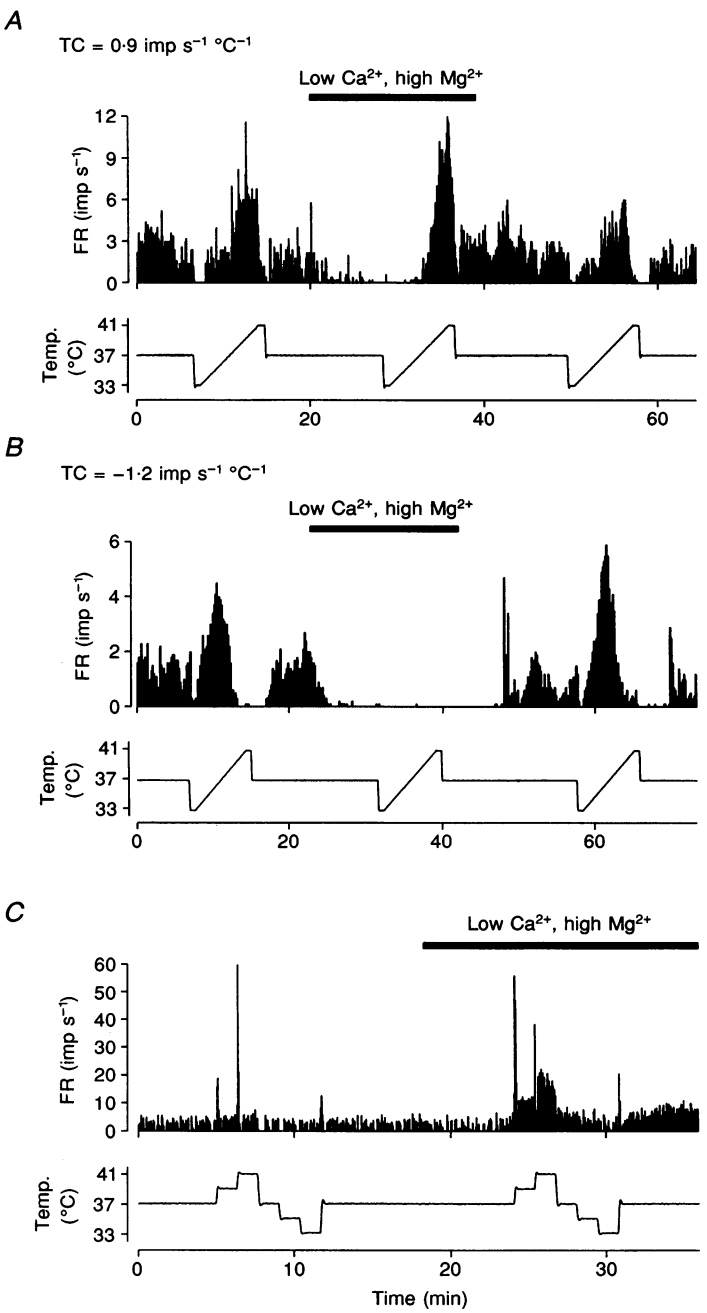

Figure 4. Effects of synaptic blockade on the temperature sensitivity and the dynamic response of SC neurones recorded from transverse slices

A, warm-sensitive lamina II neurone that retained its temperature sensitivity after synaptic transmission was blocked by superfusion of the slice with a solution of reduced Ca^{2+} (0·3 mM) and elevated Mg^{2+} (9·0 mM) concentrations. *B*, in contrast to the neurone in *A* this cold-sensitive neurone from lamina X lost its temperature sensitivity during superfusion with a low Ca^{2+}, high Mg^{2+} solution. *C*, the overshooting dynamic responses of this neurone from lamina II remained during synaptic blockade.

found. No difference with regard to the firing pattern could be observed between temperature-sensitive and -insensitive neurones. Figure 5 illustrates the characteristics of the temperature dependence of a warm-sensitive bursting neurone from laminae I and II with a TC of 3·0 imp s^{-1} $°C^{-1}$. The number of spikes per burst increased with higher temperatures, whereas the interburst intervals remained constant, as can be seen in the plots of the original impulse sequences (Fig. 5. top panel). The distribution of the shorter intervals was continuously narrowed and shifted to smaller values indicating that the intraburst intervals became shorter and more regular at higher temperatures. The intraburst intervals were reduced from approximately 4 ms at 33 °C to less than 2 ms at 41 °C. Additionally, a second population of intraburst intervals, starting at the time indicated by *b*, appeared at higher temperatures. As the interburst intervals remained unchanged, the discharge rate was essentially determined by the number of spikes per burst. The same characteristics of these temperature dependencies were observed in eight of the thirty-four warm-sensitive bursters in laminae I and II. In some of the temperature-insensitive bursters in this area, a similar temperature dependency of the intraburst intervals could be found, although they did not alter their FR in response to the temperature changes.

Dynamic responsiveness of SC neurones to rapid temperature changes

Seventy-three per cent of all tonically warm-sensitive neurones in laminae I and II ($n = 79$) responded to step-like temperature elevations with a transient increase in activity (see Table 1) and in addition, 20% of these displayed a transient inhibition during cooling. However, dynamic temperature responses were not restricted to warm-sensitive neurones, but could also be observed in 59% ($n = 90$) of the temperature-insensitive neurones in laminae I and II. Two of these neurones showed reversed dynamic responses, i.e. a dynamic increase during cooling, although in one of these a dynamic overshoot could also be elicited by rapid warming. None of the cold-sensitive neurones tested

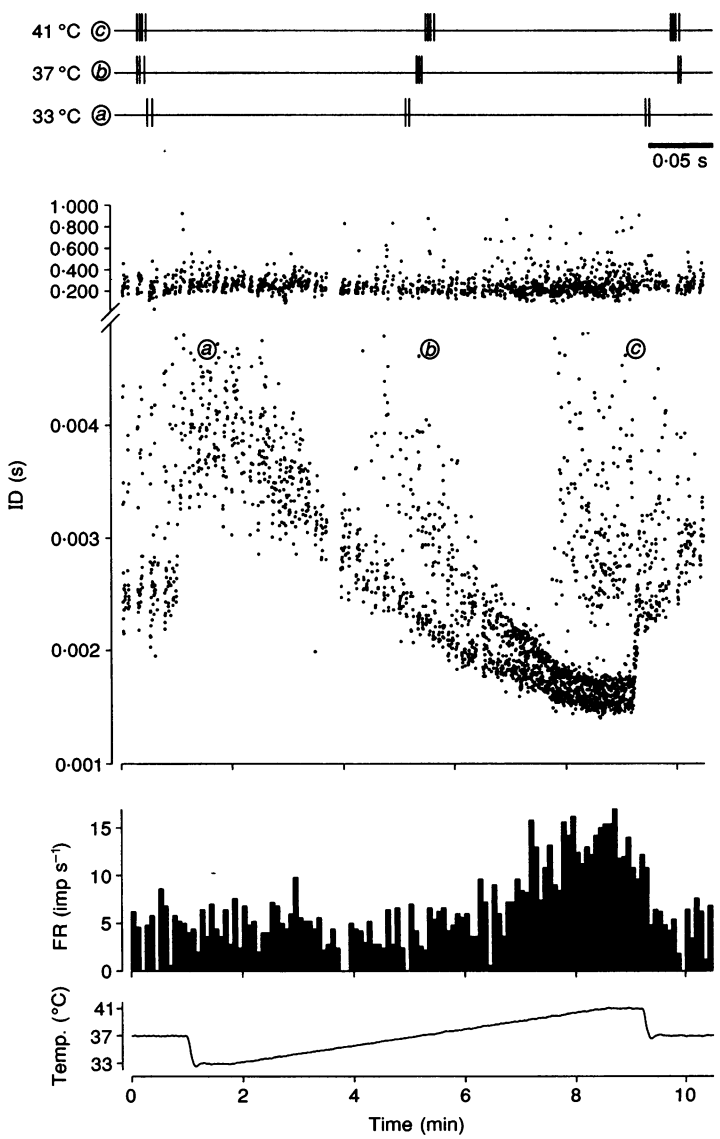

Figure 5. Modification of the bursting activity of a warm-sensitive neurone during a ramp-like temperature stimulus

The changes in the discharge rate in response to the temperature changes are displayed in the rate-meter recording with a bin width of 5 s. The modifications of the burst pattern are illustrated by means of a continuous plot of successive interspike-interval duration (ID). The increase in the number of spikes per burst at higher temperatures is illustrated in the upper part of the figure. The impulse sequences *a–c* in the upper trace are normalized spikes generated by a computer for clarity, but using the original recordings of the neurone at the times indicated in the middle plot, thus reflecting the original recordings.

in this area showed a dynamic temperature response. In lamina X only 9% of the warm-sensitive and 3% of the temperature-insensitive neurones could be classified as having a dynamic temperature response, a difference that is highly significant. One cold-sensitive neurone in this area showed dynamic overshoots in response to rapid cooling (example in Fig. 3), while the second showed a paradoxical overshoot caused by rapid warming.

Representative examples of dynamic responses elicited by step-like temperature changes are shown in Fig. 6. Rapid warming caused transient overshooting in the warm-sensitive neurone in Fig. 6*A*. The cold-sensitive neurone in Fig. 6*B* showed dynamic overshoots in response to rapid cooling. In Fig. 6*C* and *D*, two tonically temperature-insensitive neurones are shown. In contrast to the neurone in Fig. 6*C* that had a spontaneous FR and showed transient increases in activity due to rapid warming, the neurone in Fig. 6*D* had no spontaneous activity and could only be stimulated by rapid warming.

The average peak values of the transient overshoots elicited by the rapid temperature changes of 2 °C (from 37 to 39 °C and from 39 to 41 °C) were calculated for the neurones in laminae I and II using a bin width of 5 s. The mean values for the second step (39–41 °C) were significantly higher compared with the first step (37–39 °C). This was true for the warm-sensitive ($14{\cdot}8 \pm 1{\cdot}6$ and $8{\cdot}7 \pm 1{\cdot}1$ imp s^{-1}) as well as for the temperature-insensitive neurones ($9{\cdot}3 \pm 1{\cdot}2$ and $5{\cdot}6 \pm 0{\cdot}8$ imp s^{-1}). The difference in the absolute values between the warm-sensitive and temperature-insensitive neurones was obviously due to the higher FR of the warm-sensitive cells, and thus no difference in the relative values could be observed between the two groups.

In four neurones displaying a strong dynamic temperature component the effect of the rate of change in temperature on the size of the transient overshoot was tested. The dependency of the size of the overshoot on the rate of the temperature changes was the same for all these neurones and is illustrated by the example in Fig. 7. The transient overshoot decreased with the rate of temperature change and at a rate of 0·03 °C s^{-1}, no dynamic component could be elicited anymore. However, no adaptation or desensitization occurred, because the last temperature stimulus performed with the same rate of temperature change as the first elicited qualitatively and quantitatively the same dynamic response, despite the fact that the spontaneous activity of this neurone decreased slightly over time.

The spontaneous activity of those warm-sensitive neurones in laminae I and II that showed a dynamic temperature

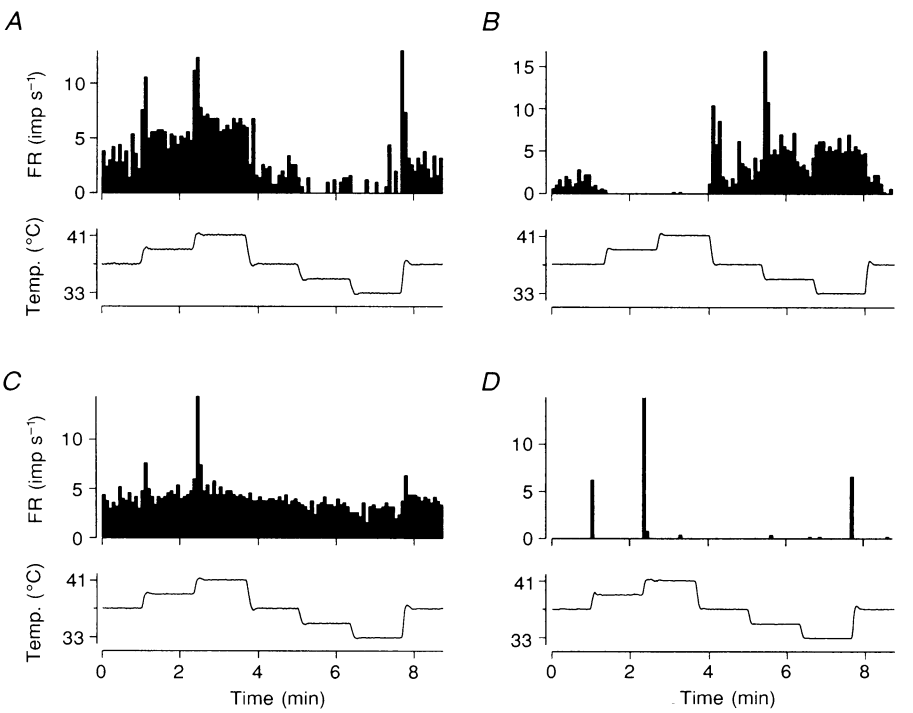

Figure 6. Dynamic temperature responses of SC neurones recorded from transverse slices

A, a tonically warm-sensitive neurone from the superficial dorsal horn showing additional dynamic responses. *B*, this cold-sensitive neurone from lamina X displays dynamic responses during rapid cooling. *C*, although this neurone from lamina II showed no tonic temperature dependence, rapid warming elicited transient overshoots. *D*, this neurone from lamina II had no spontaneous activity and responded only to rapid warming steps.

Table 2. Proportion of warm-sensitive and temperature-insensitive neurones recorded from laminae I + II of transversal slices classified according to dynamic responsiveness in each firing pattern group

	Warm sensitive		Temperature insensitive	
Firing pattern	Dynamic	Not dynamic	Dynamic	Not dynamic
Irregular (%)	71	43	71	51
Regular (%)	29	57	22	41
Silent (%)	—	—	7	8
n	56	21	54	37

response was significantly lower (mean FR = 4·8 ± 0·5 imp s^{-1}) compared with those not having a dynamic component (7·2 ± 0·9 imp s^{-1}), but there was no difference in the mean TC. No such difference in the FR existed for the tonically temperature-insensitive neurones. Table 2 shows the proportions of warm-sensitive and temperature-insensitive neurones recorded from laminae I and II according to their dynamic temperature responses and firing pattern. The number of irregular firing, warm-sensitive neurones with a dynamic temperature component was significantly higher compared with the number of warm-sensitive neurones classified as not dynamic. No difference in the proportion of temperature-insensitive neurones could be observed (Table 2).

Different types of temperature stimuli and their effect on temperature sensitivity

In order to characterize the tonic temperature sensitivity, sinusoidal and ramp-like temperature changes were used. In addition, the use of step-like temperature changes detected not only dynamic responses, but also allowed the determination of static temperature dependencies at 33, 35, 37, 39 and 41 °C. These temperature levels were maintained for 75 s. In thirty-one warm-sensitive neurones, the responses to sinusoidal and ramp-like temperature stimuli were compared. The temperature responses to the sinusoidal change of twenty-five of these neurones could be better characterized using two regression lines whereas this was the better fit for twenty responses to the ramp-like temperature

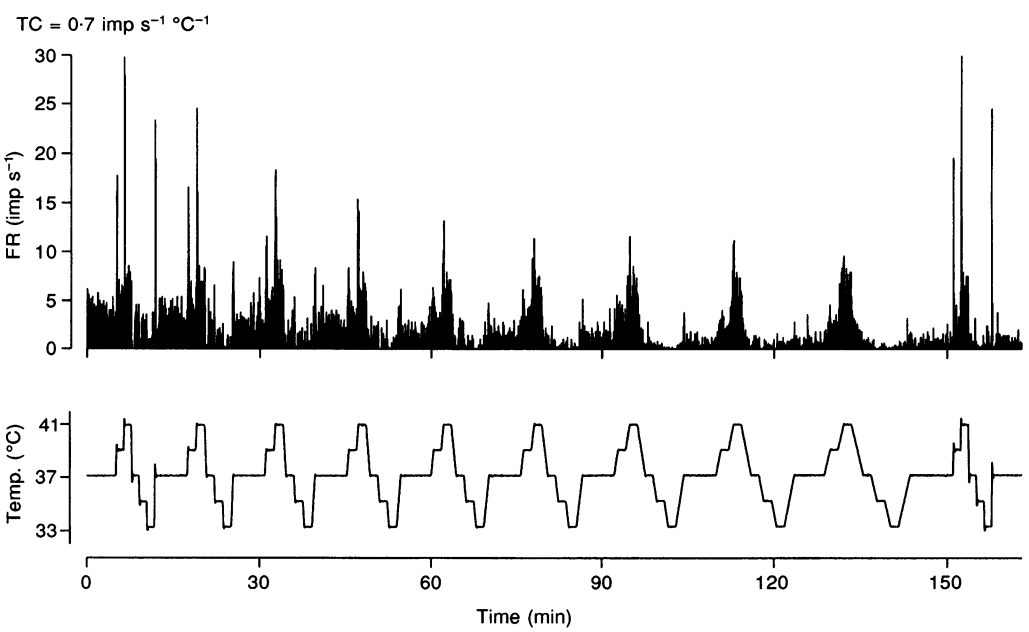

Figure 7. The size of the dynamic overshoot elicited by rapid warming is dependent on the rate of the temperature changes

The continuous rate-meter recording of this neurone from lamina II shows that the transient overshoot decreases with the rate of temperature change. Different slopes of step-like temperature changes with unchanged magnitude were applied in subsequent trials (rates of temperature change in succession: 0·4, 0·2, 0·13, 0·1, 0·08, 0·07, 0·05, 0·04, 0·03 °C s^{-1} and, as a control, 0·4 °C s^{-1} again).

changes. The temperature coefficients were significantly higher for the sinusoidal temperature stimuli (mean TC = 2·4 ± 0·3 imp s^{-1} $°C^{-1}$) as compared with the ramp-like temperature changes (mean TC, 1·6 ± 0·2 imp s^{-1} $°C^{-1}$), tested on the same cells over the same time and temperature range. When comparing only the TC of those temperature responses where the piecewise regression was used for the sinusoidal as well as for the ramp-like changes, the TC for the temperature responses to the sinus (2·8 ± 0·4 imp s^{-1} $°C^{-1}$) were still significantly higher than those for the ramp (2·0 ± 0·3 imp s^{-1} $°C^{-1}$) in the same neurones ($n = 17$), whereas there was no difference in the set-points. Figure 8 shows an example of a warm-sensitive neurone where all three temperature stimuli could be tested and demonstrates the importance of not relying on just a single type of stimulus. The frequency–temperature relationship for the sinusoidal change is described better using two regression lines, whereas for the ramp-like temperature change the linear regression was more exact. In all twenty-four warm-sensitive neurones that could be investigated using all three temperature stimuli, there was no difference in the TC between ramp- and step-like temperature changes, but the TC for the sinusoidal changes was significantly higher compared with the other two (mean TC for sinus: 2·2 ± 0·3 imp s^{-1} $°C^{-1}$; ramp, 1·6 ± 0·2 imp s^{-1} $°C^{-1}$; steps, 1·6 ± 0·2 imp s^{-1} $°C^{-1}$).

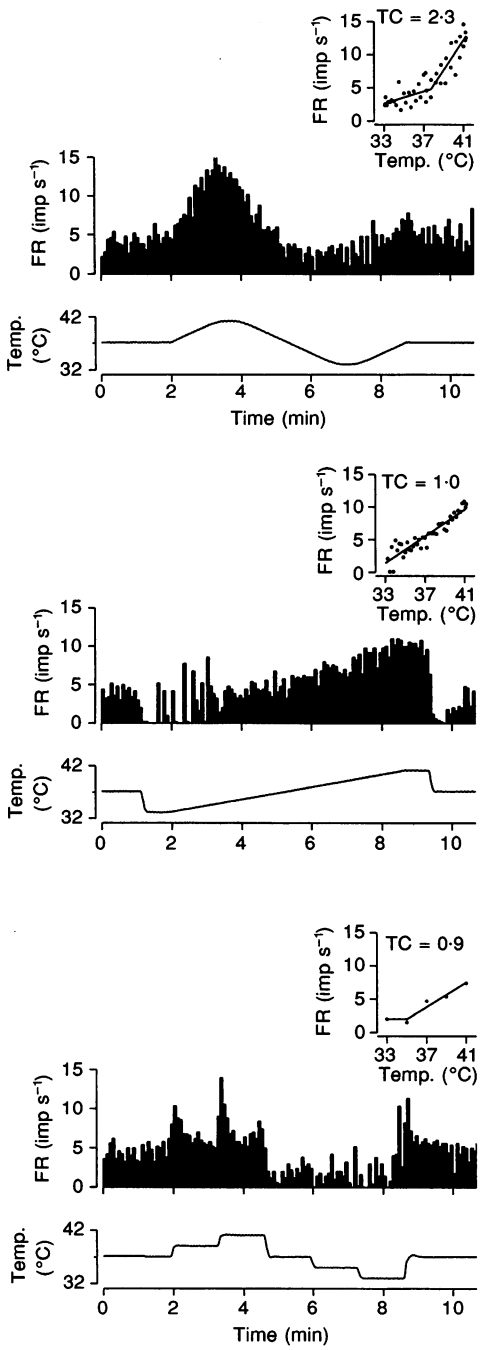

Figure 8. Responses of a warm-sensitive neurone from lamina II of a transverse slice to different types of temperature stimuli over the same temperature range

For each temperature stimulus the FR is plotted in the inset as a function of temperature. The responses to the sinusoidal temperature change (top; TC = 2·3 imp s^{-1} °C) of this neurone were greater than those to the ramp-like (middle; TC = 1·0 imp s^{-1} $°C^{-1}$) and step-like (bottom; TC = 0·9 imp s^{-1} $°C^{-1}$) temperature changes.

DISCUSSION

This study was the first in which the thermosensitivity of spontaneously active spinal cord neurones in an *in vitro* slice preparation was investigated using the methodology that has frequently been used to characterize thermosensitive neurones in the PO/AH of various homeothermic species. Studies on brain slices from the PO/AH have contributed significantly to the understanding of the cellular elements underlying the thermoregulatory heat loss and heat gain responses elicited by local warming and cooling of this brain region, which has thermosensory as well as thermointegrative functions (Hori *et al.* 1980; Kelso & Boulant, 1982; Hori, 1991; Schmid & Pierau, 1993; Boulant, 1994). Although the intrinsic thermosensory function of the spinal cord has been equally well established in *in vivo* experiments as the thermosensory function of the PO/AH (Simon, 1974; Simon, Pierau & Taylor, 1986) the location and properties of neurones responsible for the spinal thermosensitivity had not been investigated before *in vitro*. The observation that local thermosensitive neurones in the spinal cord receive specific input from peripheral temperature receptors (Simon, 1972), as well as the finding that peripheral temperature receptors terminate preferentially in laminae I and II of the spinal cord (Perl, 1990), led to the prediction that neurones located in laminae I and II are primarily responsible for the intrinsic spinal thermosensitivity. Thus the data obtained from neurones in laminae I and II are of particular interest when comparing thermosensitive SC neurones with thermosensitive PO/AH neurones. Neurones in lamina X have been shown to belong to the afferent sensory (Honda, 1985; Honda & Perl, 1985; Granum, 1986; Lee, Price, Williams, Mayer & Beitz, 1993) or efferent sympathetic system (Hancock & Peveto, 1979; Strack, Sawyer, Marubio & Loewy, 1988) and therefore their electrophysiological properties will be discussed separately.

The spontaneous activities of warm-sensitive and temperature-insensitive neurones found in laminae I and II were not different from those of warm-sensitive and temperature-insensitive PO/AH neurones recorded under conditions identical to those used in this study (Schmid & Pierau, 1993). Comparing the numbers of temperature-sensitive neurones from slices of the PO/AH and laminae I and II of the SC *in vitro* in the same two studies revealed similar percentages of warm-sensitive (46 and 53%) and cold-sensitive (5 and 2%) neurones and is well within the average number of warm-sensitive (30–60%) and cold-sensitive neurones (0–15%) generally described in studies on PO/AH slices (Boulant & Dean, 1986).

There are several reasons why the quantitative results of various studies on the thermosensitivity (in terms of TC or Q_{10}) of warm-sensitive and cold-sensitive neurones in slices of the PO/AH and SC cannot be compared directly. First, the temperature sensitivity of neurones characterized *in vitro* is affected by the extracellular Ca^{2+} concentration (Schmid & Pierau, 1993) and by the presence of certain neuropeptides (Schmid, Jansky & Pierau, 1993) and neurotransmitters (Yakimova, Sann, Schmid & Pierau, 1996) in the extracellular solution. Second, the present study has shown that the thermosensitivity determined experimentally also depends on the type of temperature stimulus used. Sinusoidal temperature changes, for example, resulted in a significantly higher TC than did ramp-like temperature changes spanning the same temperature range between 33 and 41 °C. Third, the number of temperature-sensitive neurones depends of course on the criterion used for classifying a neurone as temperature sensitive as has been pointed out before by Boulant & Dean (1986). Even though there are a wide variety of factors that may influence the thermosensitivity and thus the number of neurones regarded as temperature sensitive *in vitro*, the proportions of temperature-sensitive neurones are remarkably similar in slices of the SC and PO/AH.

In vivo studies found a relationship between cold-sensitive and warm-sensitive neurones of about 1 : 3 in the spinal cord (Simon & Iriki, 1970; Hackmann & Simon, 1975), but in the present *in vitro* study of the SC, cold-sensitive neurones were extremely scarce accounting for proportionally less than 1 : 10 in relation to warm-sensitive neurones. A similar difference in the relative frequency of warm- and cold-sensitive neurones has been observed when comparing the results of many *in vivo* and *in vitro* studies of temperature-sensitive neurones in the PO/AH (Boulant & Dean, 1986).

It has been suggested that in the hypothalamus inherent thermosensitivity might be restricted to warm-sensitive neurones; apparently cold-sensitive neurones are actually temperature-insensitive interneurones receiving inhibitory synaptic input from warm-sensitive neurones (Hammel, 1968). Since many neuronal interconnections are interrupted in transverse tissue slices, the lower number of cold-sensitive neurones in the PO/AH and SC found *in vitro* might be due to a reduction in the inhibitory synaptic input. This would be consistent with the present experiments showing that warm-sensitive neurones in SC slices generally retained their temperature sensitivity in the blocking solution, whereas the one cold-sensitive neurone tested became inactive in the blocking solution. Similar results have been obtained in studies on PO/AH slices, although exceptions have also been reported (Hori *et al.* 1980; Kelso & Boulant, 1982; Dean & Boulant, 1992), and the present result is evidence for an inherent warm sensitivity and a synaptically induced cold sensitivity of SC neurones.

In recordings from longitudinal slices of the spinal dorsal horn no cold-sensitive neurones could be found, which suggests that the cold signal in the spinal cord is not due to longitudinal intersegmental interactions, which is of course largely disrupted in transverse slices.

The finding that 73% of the warm-sensitive neurones in laminae I and II and 59% of the tonically temperature-insensitive ones there showed a dynamic thermosensitivity

is probably specific to these spinal neurones, since none of the four warm-sensitive or five temperature-insensitive PO/AH neurones from which signals were recorded in an exploratory study on slices (H. A. Schmid, unpublished observation) displayed dynamic overshoots in response to the same rapid temperature changes used in the present study (0·4 °C s^{-1}). The dynamic thermosensitivity of neurones in laminae I and II must be an inherent property of these cells, since it was preserved during synaptic blockade. *In vivo* recordings from the cervical anterolateral tract of the spinal cord during thermal stimulation of the lumbar region revealed a large proportion of thermosensitive afferent units with strong dynamic responses (Simon & Iriki, 1971*a*,*b*), and each of these temperature-sensitive units received specific input from peripheral temperature receptors (Simon, 1972). The warm-sensitive laminae I and II neurones found in the present study have properties resembling those of peripheral temperature receptors, which are also characterized by static as well as dynamic temperature sensitivities and which often show temperature-dependent bursts in firing pattern (Hensel, 1981). The previously characterized thermosensitive afferent fibres of the cervical SC (Simon & Iriki, 1971*a*,*b*) thus probably originate from somata located in laminae I and II, and these locally thermosensitive neurones receive specific input from peripheral thermoreceptors which are known to terminate predominantly in laminae I and II of the SC (Iggo & Ramsey, 1976; Kumazawa & Perl, 1978; Light, Trevino & Perl, 1979; Brown, 1982; Sugiura, Lee & Perl, 1986; Perl, 1990).

Although dynamic thermoresponsiveness alone cannot be relevant for monitoring deep-body temperature, its appearance in warm-sensitive neurones in laminae I and II but not in lamina X and in the PO/AH suggests that peripheral thermoreceptors and neurones in laminae I and II share common membrane properties. Furthermore, neurones from lamina X differed from those found in laminae I and II, with respect to a significantly higher spontaneous activity, a lower thermosensitivity in terms of Q_{10}, the rarely observed dynamic temperature sensitivity and the lower number of neurones with a burst-like firing pattern. These differences and the histological evidence that many lamina X neurones in the lumbar SC belong to the central autonomic nucleus (Hancock & Peveto, 1979; Strack *et al.* 1988) and thus serve efferent sympathetic functions suggest that neurones in laminae I and II are primarily responsible for a thermosensory function of the SC.

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Acknowledgements

The authors thank Dr H. Sann for writing the computer scripts for performing and evaluating the different temperature stimuli. This work was supported by grant Si 230/8-1 from the Deutsche Forschungsgemeinschaft.

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Received 15 April 1996; accepted 3 October 1996.