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Plaghki, Léon, Dominique Bragard, Daniel Le Bars, Jean-Claude Willer, and Jean-Marie Godfraind. Facilitation of a nociceptive flexion reflex in man by nonnoxious radiant heat produced by a laser. *J. Neurophysiol.* 79: 2557–2567, 1998. Electromyographic recordings were made in healthy volunteers from the knee-flexor biceps femoris muscle of the nociceptive R_{III} reflex elicited by electrical stimulation of the cutaneous sural nerve. The stimulus intensity was adjusted to produce a moderate pricking-pain sensation. The test responses were conditioned by a nonnoxious thermal ($\leq$40°C) stimulus applied to the receptive field of the sural nerve. This stimulus was delivered by a CO_2 laser stimulator and consisted of a 100-ms pulse of heat with a beam diameter of 20 mm. Its power was 22.7 $\pm$ 4.2 W (7.2 mJ/mm^2), and it produced a sensation of warmth. The maximum surface temperature reached at the end of the period of stimulation was calculated to be 7°C above the actual reference temperature of the skin (32°C). The interval between the laser (conditioning) and electrical (test) stimuli was varied from 50 to 3,000 ms in steps of 50 ms. It was found that the nociceptive flexion reflex was facilitated by the thermal stimulus; this modulation occurred with particular conditioning-test intervals, which peaked at 500 and 1,100 ms with an additional late, long-lasting phase between 1,600 and 2,300 ms. It was calculated that the conduction velocities of the cutaneous afferent fibers responsible for facilitating the R_{III} reflex, fell into three ranges: one corresponding to Aδ fibers (3.2 m/s) and two in the C fiber range (1.3 and 0.7 m/s). It is concluded that information emanating from warm receptors and nociceptors converges. In this respect, the present data show, for the first time, that in man, conditioning nonnociceptive warm thermoreceptive Aδ and C fibers results in an interaction at the spinal level with a nociceptive reflex. This interaction may constitute a useful means whereby signals add together to trigger flexion reflexes in defensive reactions and other basic motor behaviors. It also may contribute to hyperalgesia in inflammatory processes. The methodology used in this study appears to be a useful noninvasive tool for exploring the thermoalgesic mechanisms in both experimental and clinical situations.

INTRODUCTION

Direct investigation of neuronal convergence at the cellular level is difficult in animals and impossible in man. However, it is possible to use an indirect approach that involves testing interactions elicited by the activation of different pathways. In this respect, spatial and temporal interactions between afferent signals contributing to flexion reflexes have been studied in both animals and man (Lundberg 1979; Schomburg 1990). In all but one (Lundberg et al. 1977) of these studies, the nociceptive nature of the conditioning stimulation appeared to be the common denominator that allowed clear interactions between the afferent signals to be seen. More recently, Steffens and Schomburg (1993) showed that excitatory postsynaptic potentials evoked by cutaneous and joint afferents clearly were facilitated by conditioning radiant heat only if the stimulation temperatures were well above the pain threshold.

In animals, there are several lines of evidence that nociceptive flexor reflexes can be facilitated by homotopic noxious conditioning stimulation. Wall and Woolf (1984) reported that in the decerebrated spinal unanesthetized rat, a conditioning procedure of applying 20 electrical stimuli to the sural nerve at 1 Hz, resulted in a marked increase in the excitability of biceps femoris/semitendinosus motoneurons for 10 min. Only electrical conditioning stimuli at intensities that recruited afferent C fibers were effective in producing a prolonged facilitation of the reflex. This observation was confirmed repeatedly in the same preparation with nearly identical experimental protocols (Wiesenfeld-Hallin 1985; Wiesenfeld-Hallin et al. 1990, 1991; Woolf and Wall 1986; Woolf and Wiesenfeld-Hallin 1986). It also has been observed in spinal rabbits during recordings of reflex responses elicited in the nerves to the semitendinosus or gastrocnemius muscle by stimulation of the sural nerve. In this last case, the facilitation was seen after noxious stimulation of the heel or the application of a train of electrical stimuli to the sural nerve at an intensity that recruited Aδ and C fibers (Catley et al. 1984; Clarke et al. 1992). Long-lasting postconditioning depolarizations have been observed during recordings from motoneurons in the neonatal rat spinal cord in vitro after homosynaptic or heterosynaptic activation by repetitive stimulation of high-threshold afferent fibers (Sivilotti et al. 1993; Thompson et al. 1993). Similarly, it has been reported that in the spinal unanesthetized rat, there is a decrease in the latency for hindpaw withdrawal from noxious heat after nociceptive electrical conditioning of the sciatic nerve (Cleland et al. 1994). A decrease in the tail flick latency also has been seen after noxious thermal conditioning of the tail in pentobarbital/chloral, lightly anesthetized intact rats (Cridland and Henry 1988; Yashpal et al. 1991, 1993).

Other studies of nociceptive reflexes have shown that noxious stimuli applied homotopically can be either facilitatory as mentioned earlier or inhibitory. Indeed, strong segmental inhibitory effects have been reported after high-intensity conditioning applied ipsilaterally on or near to the same dermatome (Catley et al. 1983; Chung et al. 1983; Clarke et al. 1988, 1989; Shin et al. 1986; Taylor et al. 1989–

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1991). Interestingly, it was found that the effects found in spinal animals, whether facilitatory or inhibitory, can be modified drastically in intact nontransected animals where descending inhibitory processes are fully operational (Fleischman and Urca 1988; Gozariu et al. 1995).

It is a common observation that conditioning nonnociceptive afferents do not reveal interactions with afferent signals produced by a nociceptive test stimulus; nevertheless it is still well accepted that fibers conveying nonnociceptive information contribute to the pool of ''flexor reflex afferents'' (FRA) (Eccles and Lundberg 1959). In view of the contribution of nonnociceptive afferents to FRA, the present study was aimed at reinvestigating the possibility that nonnociceptive thermal signals may interact with nociceptive signals in man by recording a well-defined nociceptive flexion reflex, the R_{III} reflex, elicited by applying test stimuli to a cutaneous nerve (the sural nerve); using a CO_2 laser stimulator to provide nonnoxious thermal conditioning stimulation of the receptive field of this nerve; and systematically varying the delay between the conditioning and test stimuli.

The R_{III} reflex is elicited mainly by activation of Aδ-afferent fibers and represents an objective physiological index which is highly correlated with the sensation of pain produced by electrical stimulation of the sural nerve (Willer 1977, 1984; Willer and Albe-Fessard 1983).

Laser stimulation allows precise control of stimulation parameters, elicits a well-synchronized afferent volley of impulses and can produce natural and selective thermal stimulation of low-thresholds afferents. Depending on the amount of energy delivered, the CO_2 laser stimulator produces warm and/or painful sensations by direct activation of cutaneous thermoreceptors and/or nociceptors connected exclusively to Aδ and C fibers (Bromm et al. 1984; Devor et al. 1982).

In this paper, it will be shown that conditioning nonnociceptive afferents does facilitate the R_{III} flexion reflex at various conditioning-test intervals that, by use of a mathematical model can be shown to correspond to the activation of peripheral Aδ and C fibers.

METHODS

Subjects

Experiments were carried out on 15 healthy volunteers: 7 women and 8 men, mean age 26 ± 5.7 yr (range 20–45). The rules of the local ethics committee were followed. Subjects gave their informed consent after being carefully briefed on the experimental procedures and purposes. To ensure good muscle relaxation, they were installed comfortably in an armchair. They were familiarized with the perception of electrical and laser stimuli and instructed in all the usual manipulations performed during a session. They wore protective goggles in line with the guidelines for control of laser hazards. To avoid any auditory cues that may have been associated with the production of the stimuli, the subjects wore headphones (Ultramuffit, Racal Safety).

Procedure for the test R_{III} reflex

The method for eliciting and recording the nociceptive R_{III} flexion reflex from a knee-flexor muscle was based on that described previously (Willer 1984). Briefly, bipolar electrical stimuli were applied via a pair of surface electrodes placed 20 mm apart over the submalleolar pathway of the right sural nerve (Fig. 1*A*). The stimulus consisted of a volley of eight rectangular constant-current pulses, 0.5 ms in duration, delivered over 20 ms through an isolation device (Digitimer Model DS7) driven by a pulse-generator (Master-8, AMPI). The interstimulus interval varied

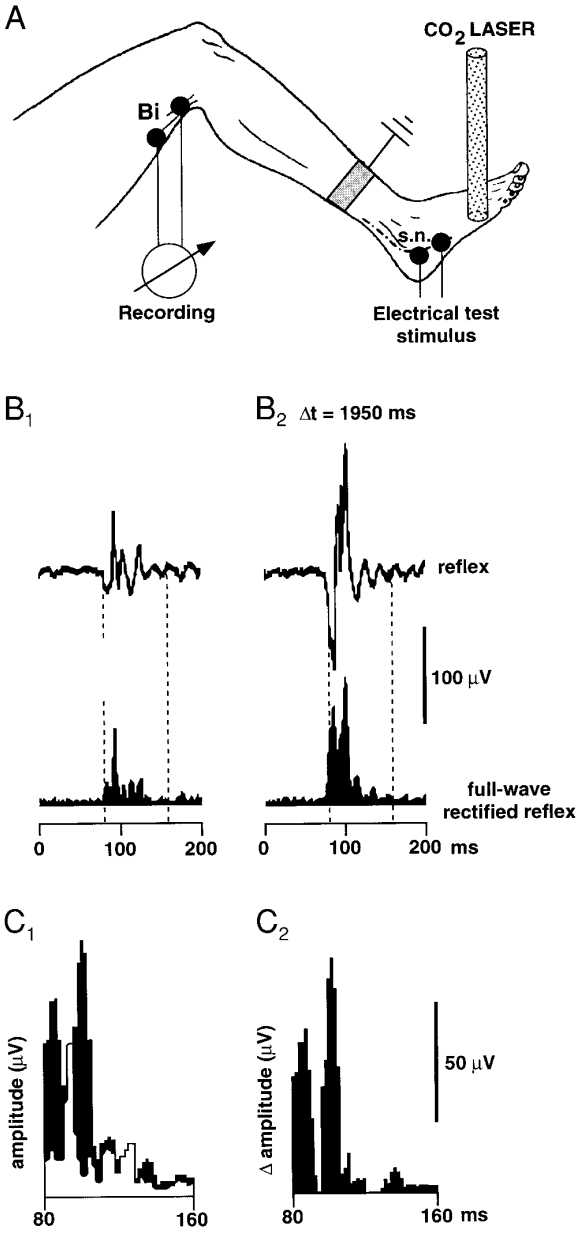

FIG. 1. Experimental setup and recording examples. *A*: experimental setup. Test R_{III} reflex was recorded from the ipsilateral biceps femoris after electrical stimulation delivered over the submalleolar path of the sural nerve at a constant intensity of 1.25 times threshold. This test electromyographic (EMG) response was or was not (controls) conditioned by a nonnoxious CO_2 laser radiant heat pulse applied to the receptive field area of the sural nerve preceding the conditioned stimulation at random interval of between 50 and 3,000 ms. *B*: example of unconditioned and conditioned R_{III} reflex responses. As shown *below* the recordings, the responses were full-wave rectified. *C*: full-wave rectified responses in the period 80–160 ms after the electrical test stimulus onset were used to provide an initial representation of the effects of the conditioning stimulus. Median of the 60 unconditioned responses then was calculated, and the individual unconditioned response closest to that value was selected, as illustrated for 1 subject in Fig. 1*C1* (□). Variations of amplitude (in V) of the EMG responses were built *(C2)* by subtracting from each bin, the median rectified unconditioned EMG response (□ in *C1*) from the individual conditioned EMG response (■ in *C1*).

from 5 to 11 s. Reflex activity evoked in the ipsilateral biceps femoris was recorded through a pair of surface Ag-AgCl electrodes (Meditrace model ECE1801) placed on the skin over the muscle, and connected to a Gould Universal Amplifier (Model 13 4615-58) with a band-pass of 10 Hz to 2 kHz and a gain of ×25,000. The upgoing edge of the electrical stimulus triggered data acquisition by a small computer (Cambridge Electronic Design Model 1401) for a period of 500 ms, at 4,000 cps. The threshold of the R_{III} reflex first was defined as the stimulus intensity eliciting liminal reflex responses with a probability of 80–90%. The current intensity was then adjusted to 25% above this threshold and was kept constant for the rest of the experiment. This intensity [mean: 14.4 ± 6.3 (SD) mA] produced supraliminal reflex responses with minimal fluctuations from one stimulus to the next (Fig. 1*B*) (Willer 1984) and a sensation of moderate pricking pain, which was well tolerated by the subjects at least for the duration of an experimental session (20 min).

Conditioning procedure

The conditioning stimulus was delivered by a CO_2 laser to the cutaneous area situated at the dorsum of the right foot and corresponding to the receptive field of the sural nerve (Fig. 1*A*). It consisted of a 100-ms duration heat pulse with a beam diameter of 20 mm and power rating of 22.7 ± 4.2 W (7.2 mJ/mm^2). With such parameters, the maximum temperature reached at the end of the stimulation period was 7°C (or 9°C with + SD of power) above the reference skin temperature, i.e., 32°C (for details of these computations, see Haimi-Cohen et al. 1983). In these conditions, the cutaneous temperature should not exceed 40°C in the superficial epidermis, and thus even the most superficial nociceptive endings should not be activated (Bromm and Treede 1983). Indeed, the stimuli were perceived as warm but never as painful. Before starting the experimental procedure, it was confirmed that the conditioning stimulus when delivered alone, did not elicit an electromyographic (EMG) response in the biceps femoris. The CO_2 laser device was an improved version of the machine conceived and built in the Department of Physics at the Université catholique de Louvain (Plaghki et al. 1989). The reproducibility of the laser stimuli was accurate at 1%. A He-Ne laser beam (Hughes Aircraft; wavelength 632.8 nm, power output 5 mW) was used to show visually the site of stimulation. The laser equipment was kept in an adjacent room to reduce noise and heat. The laser beam was deflected, through a hole in the wall, on to the foot of the subjects by means of a remotely controlled gold-coated flat mirror. The position of the laser beam was checked via a closed circuit television video; it was displaced slightly after each laser stimulus to avoid any overheating of the skin. All parameters (output power, stimulus duration, beam diameter and target site) were controlled from the experimental room.

General experimental procedure

During an experimental session, once the electrical stimulus intensity had been determined (as defined above), a total of 120 R_{III} reflex responses were studied. Sixty of these were unconditioned (control responses, i.e., those associated with a sham stimulation) and 60 were conditioned, i.e., preceded by a thermal stimulus. All these responses were elicited in a completely random order determined by a computer program. The conditioning-test interval (Δt) between the laser (conditioning) and electrical (test) stimuli was varied from 50 to 3,000 ms in steps of 50 ms.

Processing of data

The EMG records containing the reflex responses was full-wave rectified and integrated over a time window of 80 ms starting 80 ms after the test stimulus onset (Fig. 1*B*). The magnitudes of the responses (whether control or conditioned) measured in this fashion had a distribution that was skewed positively (Snedecor and Cochran 1989). In accord with the normality hypothesis being unfulfilled, medians rather than means were calculated.

The median of the 60 control responses was calculated for each of the 15 subjects. Then, the 60 conditioned responses, each corresponding to a given conditioning-test interval, and the 60 control responses were normalized to this median. This yielded for each conditioning-test interval, 15 normalized conditioned responses and 15 normalized control responses, the median of which was calculated separately and taken as the grand median of the control responses (i.e., the median of the 15 × 60 control responses). Based on these normalized data, time series vectors (control and conditioned) of equally spaced points in time (Δt = 50 ms; n = 60) were obtained and submitted to a resistant nonlinear smoothing procedure (3RSSH Tukey's notation; STATGRAPHICS, STSC) (see Figs. 2*B* and 3). This procedure of nonlinear smoothing was chosen because it is based on a ''running'' median and is resistant to ''isolated outliers.'' Because this procedure has to find the median for three values surrounding each point, the two first and two last endpoints of the times series vectors were omitted in Fig. 3.

To achieve a more comprehensible representation of data from a single subject (Fig. 2*A*), the variations in amplitude of the EMG responses were determined for the period 80–160 ms after the test stimulus; this was done by subtracting from each bin, the median value of the 60 rectified control responses from the individual conditioned response (see Fig. 1*C*).

Theoretical calculations

In this section, an attempt will be made to identify the type of peripheral nerve fibers that were activated when cutaneous sensory receptors were excited by the nonnociceptive thermal CO_2 laser stimuli. This will be done by calculating the fibers' conduction velocities by taking account of the spatio-temporal interactions, which occurred when changes in the excitability of the motoneuronal pool to the electrical test stimulus appeared after heat laser stimulation. As already shown in Fig. 3, such changes occurred only when the test response was timed properly after the conditioning stimulus.

Initial equation

During any period of facilitation of the R_{III} reflex, convergence and coincidence of information being conveyed by afferent fibers activated by the electrical test stimulus and the laser stimulus must have occurred in the CNS.

Thus for any timing of a facilitation, one can write the following equation

$$t_{pe} + t_{ae} + t_{de} + t_{cde} + (\Delta t + Z) = t_{pl} + t_{al} + t_{dl} + t_{cdl} \qquad (1)$$

where

t_{pe} = propagation time for the electrical current through the skin
t_{ae} = activation time of the nerve fibers by the electrical current
t_{de} = delay resulting from conduction time of peripheral fibers activated by the electrical current
t_{cde} = central delay time of circuits activated by the electrical current
Δt = time interval between the conditioning and the test stimulus
Z = uncertainty parameter that affects Δt
t_{pl} = propagation time of thermal energy through the superficial layers of the skin
t_{al} = activation time of sensory receptors by thermal energy
t_{dl} = delay resulting from conduction time of peripheral fibers activated by the thermal laser stimulus
t_{cdl} = central delay time of circuits activated by the thermal laser stimulus.

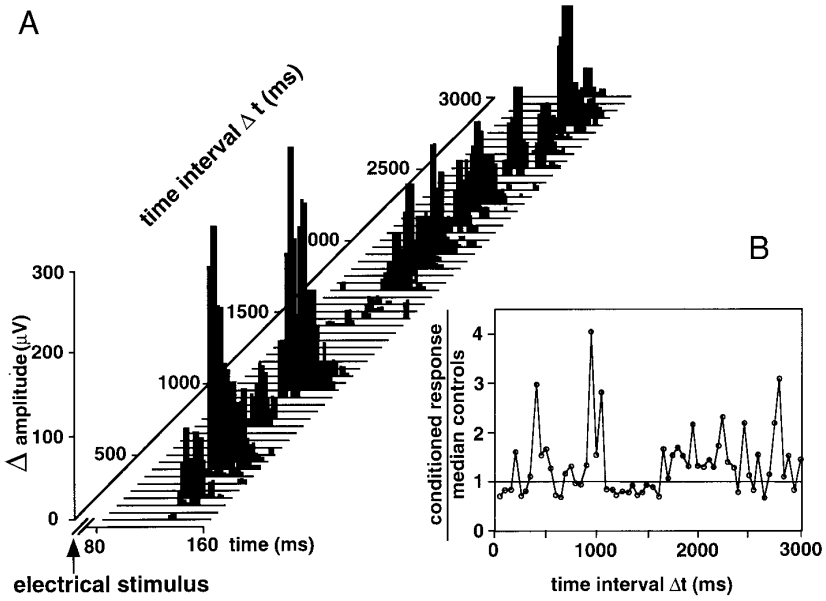

FIG. 2. Waterfall display of results obtained from 1 subject. *A*: typical example of results obtained from 1 subject (male, aged 29, stature 1.92 m) illustrating the influence of the laser thermal stimulus on the R_{III} reflex. Abscissa indicates time (in ms) with 0 corresponding to the occurrence of the test electrical stimulus; only the 80- to 160-ms time window after the stimulus is shown. Ordinate indicates the variation in amplitude (in μV) of the EMG responses by subtracting from each bin the median response of the 60 rectified control EMG responses from the individual conditioned EMG responses (see Fig. 1*C*). Sixty conditioned responses of the biceps femoris muscle are presented in a waterfall display. From *bottom* to *top*, each sweep corresponds to a response conditioned by a laser stimulus and elicited by an electrical test stimulus delivered with progressively increasing time intervals (Δt) in steps of 50 ms. *B*: graph of the ratio of the responses integrated and normalized to the median of the unconditioned responses as a function of the time interval Δt between conditioning and test stimuli. R_{III} reflex response was noticeably enhanced when intervals between the conditioning laser heat stimulus and the test electrical stimulus were ~500, 1,000, and >1,700 ms.

We will review all these parameters step by step. Because most of them could reasonably be estimated, we will propose a final *Eq. 2* giving a relationship between the experimentally controlled parameter Δt and the conduction velocities (V_l) of peripheral fibers in the sural nerve, which we presume were activated by the laser heat stimulus.

Parameters related to peripheral processes elicited by electrical stimulation

It generally is accepted that electrical stimulation directly activates peripheral nerve fibers. The propagation time of the electrical current (t_{pe}) through the skin and body fluids and the activation time (t_{ae}) of the nerve fibers both may be regarded here as negligible values.

Based on the work of Willer and Albe-Fessard (1983) and the source book of Liveson and Ma (1992), the peripheral fibers of the sural nerve involved in the R_{III} reflex are known to conduct impulses at a velocity (V_e) ~35 m/s. Because the distance of the peripheral path (D_e) may be estimated easily by measuring the distance between the stimulation site and the spinal process of T12, the peripheral conduction time (t_{de}) can be calculated as: $t_{de} = D_e/V_e$.

Parameters related to peripheral processes elicited by laser stimulation

The thermal energy sent by the laser stimulator reaches sensory receptors after a propagation time (t_{pl}) through the superficial layers of the skin and activates the receptors after an activation time (t_{al}). Their respective values presented in Table 1 are derived from the work of Haimi-Cohen et al. (1983) and of Bromm and Treede (1983, 1984).

The peripheral path distance (D_l) corresponds to the distance between the stimulation site and the spinal process of T_{12}. Unlike the electrical test stimulus, the thermal laser stimulation used in the present study did not elicit a muscle reflex; therefore, neither the conduction velocity (V_l) nor the peripheral conduction time (t_{dl}) could be measured in the present noninvasive experiments. However the relation $t_{dl} = V_l/D_l$ will be used in the calculations described later.

Parameters related to central delays

After the peripheral processes elicited by electrical stimulation, a central delay (t_{cde}) is required for related information to reach the motoneuronal pool and trigger activation of the biceps femoris muscle. After the peripheral processes elicited by laser stimulation, a central delay (t_{cdl}) also is required for related information to reach the motoneuronal pool and influence its excitability. If these central delays are equal, then these two parameters cancel each other in *Eq. 1*. If the values of these parameters are not equal, their difference $\Delta t_d = |t_{cde} - t_{cdl}|$ should be taken into account. Such a difference could derive from the propagation of action potentials through different neuronal networks, one of these possibly including additional synapses. We decided to take into account a greatly overestimated possible difference of 20 synapses. In fact, taking into account a difference of ≤20 synapses, computational analysis based on *Eq. 2* showed that this factor was of limited importance with regards to the other parameters.

Time elapsed between conditioning and test stimuli

The parameter that has been manipulated experimentally is Δt, i.e., the time elapsed between application of conditioning and test stimuli. More particularly, Δt was the time elapsed between the beginning of the 100-ms thermal stimulus and the beginning of the 20-ms train of electrical pulses. However, because the exact timing of the start of nerve fiber excitation may have varied during the stimulating period, the time elapsed between the actual effective conditioning and the test stimuli also may have varied. This uncertainty is represented by the parameter Z. According to the durations of both thermal and electrical stimuli, the ($\Delta t + Z$) value may vary from (Δt − 100 ms) to (Δt + 20 ms).

Final equation and computations

By rearranging *Eq. 1*, and applying the simplifications mentioned above, it may be rewritten

$$V_l = D_l/(\Delta t + Z + t_{de} - t_{pl} - t_{al} + \Delta_{td}) \quad (2)$$

Based on *Eq. 2*, the relation between V_l and Δt may be examined for the three sets of parameter values listed in Table 1. These values were chosen on the basis of the above-mentioned reports in the literature and taking into account under- and overestimations.

Results of the computational analysis are presented in Fig. 4. Three curves were calculated using underestimated, averaged, and overestimated parameters, respectively, as shown in Table 1. For Δt values >1 s, no significant differences were seen among the

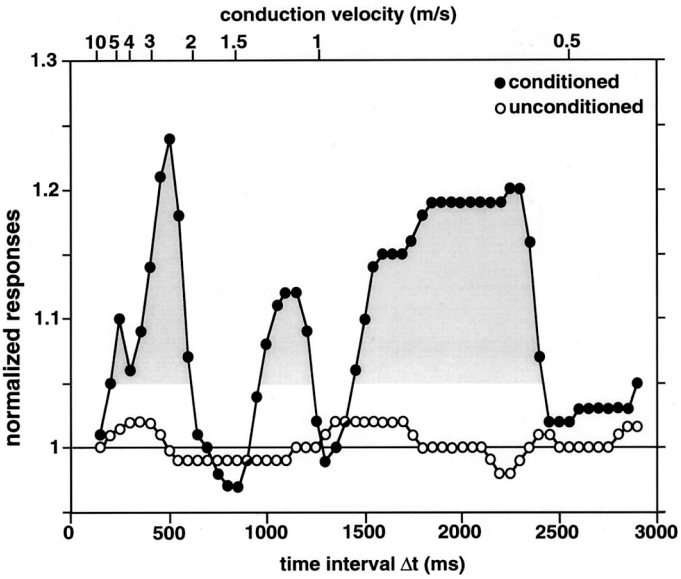

FIG. 3. Modulation of R_{III} reflex responses by conditioning nonnoxious heat stimulation. Influence of the conditioning laser thermal stimulus on the nociceptive reflex R_{III} shown for data pooled from all 15 subjects. Curve with filled circles represents the data of the smoothed normalized EMG evoked reflex activities (see METHODS) as a function of the time interval Δt between the conditioning laser stimulus and the test electrical stimulus. Curve with open circles represents the smoothed normalized data computed from the unconditioned responses, i.e., those associated with a sham stimulation. Two curves are clearly different. As compared with the controls, the curve obtained from the conditioned EMG responses shows 3 peaks at 500 ms (with the fastest conditioning effect noticed ~250 ms), 1,100 ms, and a late, large peak between 1,600 and 2,300 ms. Mean conduction velocities of any peripheral fibers excited by a conditioning stimulus when convergence and coincidence of the information conveyed by the afferent fibers activated by the test and the conditioning stimulus occurred, were computed as in Fig. 4 (*top*). Δt values corresponding to the 3 peaks matched the criteria defined by *Eq. 2* and Fig. 4: 1 in the range of Aδ fibers and 2 in the range of C fibers (see also Table 2).

three curves (Fig. 4, *front panel*). By contrast, for Δt values <500 ms, and much more dramatically for Δt values <250 ms, differences between the curves were greatly increased at decreasing values of Δt (Fig. 4, *inset*). Thus *Eq. 2* yields results that make sense from a biological point of view only for $(\Delta t + Z + t_{de} - t_{pl} - t_{al} + \Delta_{td}) \gg 0$. Indeed, the sum of the parameter values $(Z + t_{de} - t_{pl} - t_{al} + \Delta_{td})$ equal + 26, −100, and −180 ms, respectively, for the under-, mean, and overestimated parameters (Table 1). As a consequence, when using the mean and overestimated parameter values respectively, computing the conduction velocity for Δt lower than ~150 and 250 ms yields inconsistent figures.

For the Δt values corresponding to the peaks illustrated in Fig. 3, i.e., corresponding to a facilitation of the electrically induced nociceptive reflex, conduction velocities of the peripheral fibers excited by the thermal laser stimulus can be computed from the three curves shown in Fig. 4. The results are shown in Table 2. In addition, the conduction velocities of the peripheral fibers excited by the thermal laser stimulus, as estimated from the curve calculated with averaged parameters, are reported on the *top abscissa* of Fig. 3 for graphic examination. The facilitations elicited when Δt values were ~500 ms (with the fastest conditioning effect starting ~250 ms), correspond to laser-induced activities in small-diameter myelinated fibers of the Aδ type. The facilitations observed for Δt values ~1,100 ms and those ~1,600–2,300 ms, result from laser-induced activities in thin nonmyelinated nerve fibers of the C type; in addition, two separate C-fiber groups could be defined: the first with conduction velocities of 1–1.5 m/s (C1), and the second with conduction velocities <1 m/s (C2).

RESULTS

Experimental observations

In the 15 healthy volunteers, we examined whether nonnoxious radiant heat produced by a laser could influence the R_{III} flexion reflex elicited by nociceptive electrical stimulation of the sural nerve (Fig. 1*A*). The thermal stimulus was perceived by the subjects as warm but never painful. When applied alone, it never elicited an EMG response in the biceps femoris.

As illustrated with an individual example (Fig. 1*B*), the application of the laser stimulus with a 1,950-ms conditioning-test interval resulted in a clear facilitation of the R_{III} response (Fig. 1*B2*) as compared with the control response (Fig. 1*B1*). Further processing of the data involved both responses being full-wave rectified (Fig. 1*B*, *bottom*) and considered within the period 80–160 ms after the test stimulus onset. The median of the 60 unconditioned responses then was calculated, and the individual unconditioned response closest to that value was selected, as illustrated for one subject in Fig. 1*C1* (□). Finally, the variations in amplitude of each conditioned EMG response were computed by subtracting from each bin, the value of the median rectified unconditioned response from the individual rectified conditioned response (Fig. 1*C2*).

Figure 2*A* shows a typical example of the result obtained in this way from one subject, illustrating the influence of the laser heat stimulus on the R_{III} reflex. This figure was constructed by sequentially displaying the increased reflex activity (ordinate, Δ amplitude) elicited by the conditioning thermal stimulus, with the time after the test stimulus running from *left* to *right* (abscissa) and the conditioning-test intervals (Δt) increasing from *front* to *back* (z axis). The facilitation of the 60 conditioned reflex responses are illustrated in perspective using a waterfall display presentation. The data, initially acquired with conditioning-test intervals applied at random in the 50–3,000 ms range, were rearranged in such a way that each sweep of the figure corresponds to a test response conditioned by a laser stimulus with progressively increasing conditioning-test intervals from bottom to top in 50-ms steps. Note the facilitation of the R_{III} reflex for some conditioning-test intervals, separated by periods of ''silence'' during which individual EMG responses were nearly identical to the control R_{III} reflex.

The results obtained from this individual subject are shown quantitatively in Fig. 2*B*. The results are expressed in terms of the ratio (ordinate) between each individual response and the median unconditioned response as a function of the conditioning-test interval (abscissa). Both response values were calculated during the 80–160 ms after the test stimulus. The curves were obtained after a resistant

TABLE 1. *Parameter values of the model*

Parameters	Underestimated	Average	Overestimated
t_{pl}, ms	20	35	50
t_{al}, ms	20	35	50
D_l, mm	1,210	1,280	1,350
D_e, mm	1,080	1,150	1,220
V_e, m/s	30	35	40
Δt_d, ms	10	0	−10
Z, ms	20	−60	−100

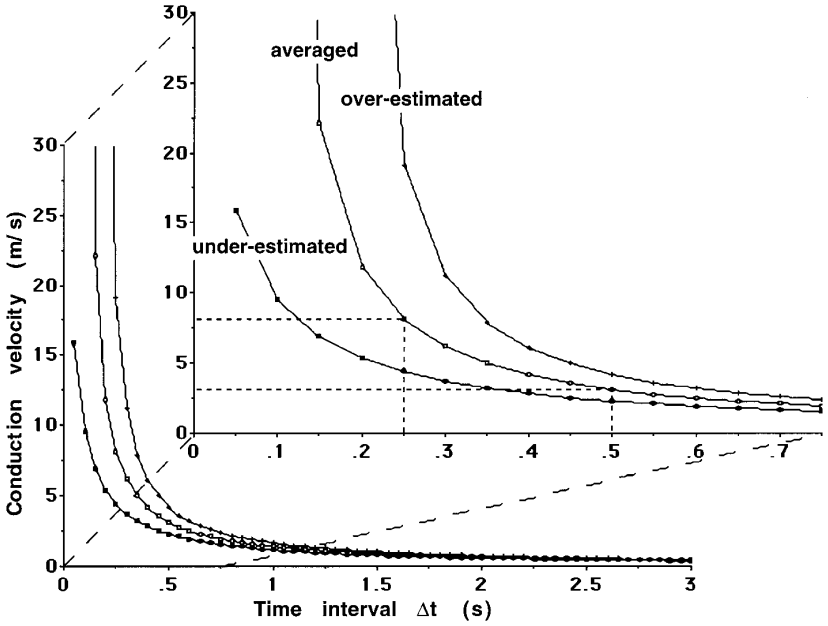

FIG. 4. Computation of the conduction velocity of primary afferent fibers engaged in convergence. Theoretical relationship between the conditioning-test interval Δt and the conduction velocity V_l of any peripheral fibers excited by a conditioning stimulus when convergence and coincidence of the information conveyed by the afferent fibers activated by the test and the conditioning stimulus occurred. By computation of *Eq. 2*, $V_l = D_l/(\Delta t + Z + t_{de} - t_{pl} - t_{al} + \Delta_{td})$, 3 curves were obtained using the underestimated, averaged, and overestimated values of the parameters, respectively, as listed in Table 1. First part of the curves corresponding to the lower Δt values (0- to 0.75-s range), are shown at the back with a higher magnification of the abscissa. Calculated values of V_l were added as the *top abscissa* in Fig. 3.

nonlinear smoothing treatment. Note that the R_{III} reflex clearly was enhanced when the conditioning-test intervals were ~500 and 1,000 ms and when they were >1,700 ms.

The profile of this curve based on a single subject (Fig. 2*B*) was similar to that for data pooled from the 15 subjects. Figure 3 shows two curves obtained after the resistant nonlinear smoothing treatment of the normalized conditioned and unconditioned responses (see *Processing data*). The curve with filled circles represents the data of the smoothed normalized EMG evoked activities as a function of the interval between the conditioning laser stimulus and the test electrical stimulus. The curve with open circles represents the smoothed normalized data computed from the unconditioned responses. The two curves are clearly different. The curve obtained from the conditioned EMG responses shows three peaks at conditioning-test intervals of 500 and 1,100 ms and between 1,600 and 2,300 ms. These peak values, together with other parameters were used to compute the conduction velocity of the cutaneous afferent fibers activated by the conditioning laser heat stimulus (see *Theoretical calculations*). In Fig. 3, the *top abscissa* shows the computed conduction velocity plotted against the corresponding EMG data. Based on the computed conduction velocity, three different groups of afferent fibers were recognized: one in the Aδ-fiber range and two in the C-fiber range (see Table 2).

DISCUSSION

It was found that the nociceptive flexion reflex was facilitated by nonnoxious thermal stimulation applied to the peripheral receptive field of the sural nerve. Such a modulation occurred with definite time intervals between the conditioning and test stimuli, namely ~500, ~1,100 ms, and a late, long-lasting phase between 1,600 and 2,300 ms. The simplest interpretation of our observations is that nociceptive and nonnociceptive information converges and interacts on a common pool of interneurons. The occurrence of such interactions implies a temporal coincidence of information emanating from the two areas of stimulation. This was obvious only with adequate time intervals between the conditioning and test stimuli. Because the R_{III} reflex is present in patients with total sections of the spinal cord (Roby-Brami et al. 1987; Willer and Bussel 1980), one can assume that its basic pathway is mainly spinal. Thus the most parsimonious interpretation of our present data is that the convergence between noxious and nonnoxious signals occurred in the spinal cord with the various peaks of facilitation being determined mainly by the time required for the synchronized peripheral volleys elicited by the heat stimulus to reach the common final pool of interneurons. Therefore the different facilitatory waves can be explained by the triggering of activity in fibers with different peripheral conduction velocities.

In spite of the parsimony of our interpretation of the present results, three other alternative interpretations could be put forward: sensitization, variation in central delays, and oscillations of cord excitability. Sensitization (see references in Meyer et al. 1994) is unlikely to introduce a bias in the present results for two reasons. First, the position of the laser beam was moved slightly after each laser stimulus to avoid any skin damage due to repetitive stimulation. Second, the stimulus intervals were applied randomly, and thus the rank order of the applied stimulus did not appear to be a significant source of variability. Variations in central delays may result from additional synapses in segmental networks and/or in long loops. These variations appear to be of limited importance given that the introduction of an uncertainty factor as large as 20 ms in the mathematical model (see *Z* parameter in *Theoretical calculations*) had little impact on the determination of the conduction velocities. Finally, it

TABLE 2. *Conduction velocities of peripheral nerve fibers*

Δt, s	Under-estimated	Mean	Over-estimated	Fiber Type
0.50	2.3	3.2	4.2	Aδ
1.10	1.1	1.3	1.5	C
1.60–2.30	0.6	0.7	0.8	C

Velocities are in meters per second.

could be questioned whether the three peaks do reflect oscillations of cord excitability triggered by the conditioning laser stimulus. Indeed mechanisms proposed to play a major role during spinal processing (convergence, temporal, and/or spatial summation and assembly coding) may depend on neuronal discharge synchronization. In this line, it has been reported that oscillations arise locally in the dorsal horn of the spinal cord and induce cooperative activity of spatially distributed neuronal subpopulations (Eblen-Zajjur and Sandkühler 1997). Dorsal root stimulation also has been shown to trigger oscillations in the motoneuron pool (Baranauskas and Nistri 1995) with a power spectrum peaking at 8 Hz. However, power spectrum of the present data peak <2 Hz. As no such low-frequency oscillator has been observed, our proposition seems more likely. Higher frequencies of oscillations, if present, should have been observed in our data given the Δt of sampling rate of 50 ms. Besides, radiant heat stimulation is known to activate different types of peripheral nerve fibers in the Aδ and C range (references below).

The peak values in Fig. 3, together with the mathematical modeling, helped us to compute the conduction velocities of the cutaneous afferent fibers activated by the conditioning thermal stimulus. Three different groups of afferent fibers were identified: one in the Aδ-fiber range (3.2 m/s) and two in the C-fiber range (1.3 and 0.7 m/s). This is consistent with the fact that the CO_2 laser stimulator activates exclusively Aδ and C fibers (Bromm et al. 1984; Devor et al. 1982) and evokes sensations through direct activation of cutaneous thermoreceptors and/or nociceptors, depending on the amount of energy delivered.

The discussion will be organized into several sections as follows: facilitation of a flexion reflex by thermal stimuli; putative peripheral fibers involved in the facilitation; putative spinal neurons involved in the facilitation; and methodological considerations.

Facilitation of a flexion reflex by thermal nonnoxious or noxious stimuli

To the best of our knowledge, there have been only two studies that have used an experimental design close to that used in the present work and that merit consideration.

Andersen et al. (1994) reported that a reflex response from the tibialis anterior muscle was facilitated by a painful thermal conditioning stimulus applied to the sole of the ipsilateral foot with conditioning-test intervals from 600 to 3,000 ms. This effect was attributed to fibers with a broadband of conduction velocities within the C-fiber population being activated by the conditioning stimulus. However, because these authors used seven fixed conditioning-test intervals (0, 300, 600, 800, 1,000, 1,200, and 3,000 ms), it was quite impossible to differentiate with accuracy several peaks of facilitatory effect as we did here by considering 60 conditioning-test intervals between the laser and electrical stimuli. Besides these differences in the resolution of conditioning-test intervals, there were others between the present study and that work, notably because in the latter EMG reflex activity was elicited from the tibialis anterior muscle by electrical stimulation of the sole of the foot whereas we recorded a well-defined nociceptive flexion reflex from the biceps femoris muscle elicited by stimulation of Aδ- fibers in a cutaneous nerve, the sural nerve; because the conditioning stimulation was provided by a Xenon lamp (visible light) and was noxious whereas in our study, a mild sensation of warmth was described by the subjects; and because the test stimulus was applied to the sole of the foot, whereas in our study it was applied on the dorsum of the foot, i.e., within the receptive field of the sural nerve. All these differences make comparison between the present study and that of Andersen et al. (1994) quite difficult.

In the second study (Willer and Albe-Fessard 1983), the R_{III} reflex was conditioned by painful electrical stimuli (30-mA intensity, 1-ms duration) applied to the sural nerve territory. Facilitations of the R_{III} reflex were induced by such conditioning stimuli and were attributed to the activation of afferent fibers with conduction velocities in the 0.8–25 m/s range. In this case, however, the conditioning stimulus was also nociceptive in nature, and facilitations were in the 150–200% range. By contrast, the moderate facilitations (15–20%) observed in the present study were elicited by nonnoxious conditioning radiant heat. In any case, as far as we know, the facilitation of nociceptive reflexes by nonnoxious thermal stimuli has not been reported to date.

Putative peripheral fibers involved in the facilitation

It was possible to subdivide into three groups, the peripheral fibers which when activated facilitated the R_{III} reflex because of the time resolution resulting from the experimental design. However, as suggested in Fig. 3, the first peak of activation might in fact be composed of two components, both in the range of Aδ-fiber conduction velocities. However, before a definite conclusion can be made about this, further experiments involving shorter time intervals (Δt) between the test and conditioning stimuli will be required.

It should be emphasized that the three groups of fibers that we are proposing were involved in the modulation of the R_{III} reflex, conveyed information subserving warm perception. This is supported not only by the verbal reports of the subjects but also by the computation of maximal temperatures reached in the center of the laser beam, which were always <40°C (see *Conditioning procedures*).

The responses of peripheral receptors and afferent fibers to thermal stimulation have been studied widely with microneurographic techniques (e.g., Beck et al. 1974; Vallbo et al. 1979; Van Hees and Gybels 1972). Several classes of cutaneous receptors that may respond to thermal stimuli are connected to slowly conducting thin fibers, either myelinated (Aδ) or unmyelinated (C). The afferent fibers that selectively respond to thermal stimuli generally are classified either as cold receptors, connected to Aδ fibers or warm receptors, connected to C fibers. This dichotomy is based mainly on the observation that warm sensibility is blocked earlier than cold sensibility after local anesthesia (Fruhstorfer et al. 1974; Sinclair and Hinshaw 1950). However, this separation between Aδ cold and C warm receptors is not absolute (Spray 1986) and, as discussed later, several types of peripheral receptors might have contributed to the results reported herein.

In the myelinated afferent fiber group, the Aδ cold receptors are known to operate optimally in the 15–30°C range. In the monkey, the mean conduction velocity of these afferent fibers is 9.0 m/s with a large range from 1.7 to 16.7 m/s (Dubner et al. 1975). These receptors present resting

discharges at ≤11 Hz at the optimal temperature of 25°C and disappear beyond 38°C (Beitel et al. 1977; Darian-Smith et al. 1973; Sumino and Dubner 1981). Such a decreased activity of the Aδ-cold receptors in the 25–38°C range makes the participation of this class of neurons unlikely in the facilitation of the R_{III} reflex.

Observations of Aδ-fiber activity associated with warm perception have been reported but the proportion of such afferent fibers is low compared with cold receptors (Darian-Smith et al. 1973). In the monkey, facial warm receptors connected to Aδ and C fibers with conduction velocities in the 0.9–6.7 m/s range have been reported (Sumino et al. 1973). Interestingly, the warm receptors described by Terashima and Liang (1993) in man, are linked to Aδ fibers with conduction velocities in the 3.8–11.2 m/s range. These conduction velocities are reasonably close to those computed in the present study (3.1–8.1 m/s); such fibers may have contributed to the early facilitation of the R_{III} reflex reported herein.

A priori, two other types of Aδ receptors also might have contributed to these effects. In the first subclass, the A-HTM (high-threshold mechanical) or type I A-MH (mechano-heat), the Aδ receptors respond to strong mechanical stimulation and usually remain insensitive to noxious heat and chemical stimulation. They have high thermal thresholds (>49°C) and sometimes rapid conduction velocities (mean 31 m/s, range 5–53 m/s) (Campbell et al. 1979). In the second subclass, the mechano-thermal receptors (MTR) or type II A-MH, the Aδ- receptors respond to strong mechanical stimuli, noxious heating, and algogenic chemicals. They present lower thermal thresholds (range: 37–47°C) and slower conduction velocities (mean: 15.2 ± 9.9 m/s) (Dubner et al. 1977). Although the former group is unlikely to have contributed to the early facilitation of the R_{III} reflex reported herein, the latter might have contributed to some extent.

Regarding the unmyelinated fibers, two classes of receptors are concerned with warm stimuli. In the first, the C-warm receptors are included in afferent fibers that respond optimally to temperatures ~40°C, i.e., temperatures that generally are perceived as gentle warmth. Their conduction velocities are reported to be between 0.6 and 2.4 m/s, with mean values between 0.7 and 1.2 m/s (Darian-Smith et al. 1979; Duclaux and Kenshalo 1980; Hensel and Iggo 1971; LaMotte and Campbell 1978); these are reasonably close to those computed in the present study (0.7–1.3 m/s). Such receptors were therefore probably involved in the late facilitations of the R_{III} reflex reported herein.

In the second class, the C-mechano–heat nociceptors (C-MHs), the unmyelinated afferent fibers respond to high-intensity mechanical and thermal stimuli. They were shown to sometimes respond to chemical stimuli and thus also are termed polymodal. Their thermal threshold ranges between 38 and 49°C with mean values ~44°C (Beitel and Dubner 1976; Robinson et al. 1983). They show no spontaneous activity below 38°C (Hallin et al. 1981; LaMotte and Campbell 1978). In humans, the conduction velocity for this group is reported to be in the 0.7–1.3 m/s range with mean values ~0.8 m/s (Gybels et al. 1979; Van Hees and Gybels 1972). Even though their participation cannot be ruled out completely, it is unlikely that such fibers would have contributed significantly to the thermally induced late facilitations of the R_{III} reflex.

Although the distribution of C-fiber conduction velocities is most commonly said to be unimodal, it was found to be bimodal in the present study. As far as we know, the existence of two groups of conduction velocities of C fibers was reported by Douglass et Ritchie (1959). However, these C fibers were not sensitive to nonnoxious warm stimulation. In a study of C-fiber warm receptors, Darian-Smith et al. (1979) provided a histogram of the distribution of their conduction velocities: it was clearly unimodal centered on 1.2 m/s. Two distinct classes of C-MH receptors were described in the monkey by Meyer and Campbell (1981) with either quickly or slowly adapting responses. However these authors do not rule out the possibility that this difference depended on the depth below the skin surface at which the receptor was located. Interestingly, latency distributions of C-fiber–evoked reflexes elicited by electrical stimulation of the sural nerve in the rat are bimodal, suggesting existence of two populations of C fibers with different conduction velocities (0.6 and 0.9 m/s) (see Falinower et al. 1994). Similar response latencies with a bimodal distribution also have been observed during recordings of rat dorsal horn convergent neurons after electrical stimulation of their receptive fields (e.g., see Villanueva et al. 1986; this study).

Putative spinal neurons involved in the facilitation

As mentioned already, the convergence of afferent signals evoked by the nonnoxious thermal conditioning and the nociceptive test stimuli most probably occurred in the spinal cord. For many reasons, such a convergence is not conceivable at the level of the motoneuronal pool responsible for the activation of the biceps femoris muscle. Radiant heat below the threshold for nociceptive afferent fibers did not elicit synaptic effects in α motoneurons nor facilitation on reflex pathways from Group Ib or II muscle afferents or mechanoreceptive cutaneous afferents (Behrends et al. 1983; Burke et al. 1971; Steffens and Schomburg 1993). Because all Aδ and C fibers terminate in the dorsal horn of the spinal cord, it follows that the functional site of convergence was located on interneurons situated upstream of the motoneuronal pool.

One subpopulation of dorsal horn neurons fulfills some of the properties required for being the locus of convergence, and several pieces of evidence suggest that they may well belong to the reflex arc involved in the R_{III} reflex. Indeed, the R_{III} reflex recorded in humans and the convergent neurons recorded in animals are modulated by common factors: both are inhibited by systemic or intrathecal morphine (see ref. in Willer and Le Bars 1995); both are inhibited by transcutaneous electrical nerve stimulation (Handwerker et al. 1975; Rosenberg et al. 1996); both are inhibited by heterotopic noxious stimuli (see references in Willer et al. 1989) via supraspinal structures located in the lower medulla (Bouhassira et al. 1995; De Broucker et al. 1990). A subset of such neurons, the so-called ''warming/noxious heat-convergent units'' have thresholds mainly <42°C, increase their discharge over a limited range of temperatures and most commonly reach a plateau or even decline slightly when noxious temperatures are achieved (Burton 1975; Kanui 1988; Le Bars and Chitour 1983; Menétrey et al. 1979; Price and

Browe 1975). This subset of neurons may well be a candidate as the locus of convergence. However, involvement of ventral horn interneurons cannot be excluded because temperature-sensitive neurons have been described within laminae VII and VIII (Burton 1975).

Methodological considerations

We believe that the present results provide and validate a potentially useful noninvasive method not only for further studies concerned with central interactions among information from various origins but also for those studies that will deal with the indirect study of peripheral fibers in man. It is clear that a certain degree of convergence is implied within the pathways underlying the R_{III} reflex. Admittedly, absence of facilitation by no means indicates absence of fiber activation by the conditioning stimulus; but occurrence of facilitation clearly indicates that a group of fibers actually have been activated by the conditioning stimulus. As quoted by Schomburg (1990) ''spatial facilitation depends on a distinct subliminal fringe around the discharge zone in the interneuronal pool shared by the two pathways.'' Thus being aware of the possible pitfalls of false negative results and considering the difficulty of investigating, in man, peripheral fibers with slow conduction velocities, the experimental method used here may be a useful tool in both experimental and clinical situations. In this respect, it is important to note that the routine electrophysiological methods used in clinical departments investigate transmission only in afferent fibers with the largest diameter and cannot explore the thermoalgesic system (Noël and Desmedt 1975; Veilleux and Stevens 1987).

We will comment briefly on some possible functional implications of the present results. Under the term ''erythralgia,'' Lewis (1942) described temperature-dependent pain elicited by experimentally induced local redness and tenderness of the skin. Using an in vitro testis-superior spermatic nerve preparation, Kumazawa et al. (1987) provided convincing evidence that such observations could be explained by a potentiation of the response of polymodal nociceptors by increases of temperature in the nonnoxious 34–43°C range (at least as far as the viscera are concerned). Because inflammation is associated with local increases in temperature, this may contribute to clinical hyperalgesia. However, as shown here for cutaneous stimuli, nonnoxious increases in temperature also could result in a central booster by means of convergence between information emanating from warm and nociceptive receptors. Together with the effects of the ''inflammatory soup'' (see reference in Dray 1994; Levine and Taiwo 1994), such factors potentially could contribute to the hyperalgesia seen during inflammatory processes.

In conclusion, the present data suggest indirectly that warm nonnociceptive information can be conveyed by both Aδ- and C-afferent fibers and can modulate the R_{III} reflex in man. Although these effects were moderate, in the 15–20% range, they do suggest that such warm afferents may contribute to some extent to the FRA and add a useful signal to that of other information to trigger flexion reflexes in defensive reactions and other basic motor behaviors.

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