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An inhibitory interaction of human cortical responses to stimuli preferentially exciting A δ or C fibers

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Abstract

Finely myelinated (type A δ) and unmyelinated (type C) fibers are the major afferent inputs to spinothalamic tract neurons mediating sensory and reflex responses to noxious and thermal stimuli. These two fiber types differ in their sensory and biophysical properties, raising questions about the interaction of their supraspinal responses. Therefore, we investigated the interaction of cortical responses to stimuli that preferentially excite these fibers in human subjects using evoked potential recordings in a paired conditioning stimulation (CS) and test stimulation (TS) paradigm. There were two experiments, one with A δ as CS and C as TS (A δ -C) and another with these stimuli reversed (C-A δ). We used intra-epidermal electrical pulses applied to the dorsal left hand at 2 and 1 $\times$ pinprick threshold (pp) for the preferential stimulation of A δ fibers and 37 – 50°C contact heat pulses applied to the left or right thenar and left hypothenar eminences for the preferential stimulation of C fibers. We found that the cortical response to preferential A δ or C fiber stimulation was attenuated whenever either cortical response preceded the other. Standardized values of peak and integrated amplitudes were < 1 in all pairing conditions and in all subjects in both experiments. The suppressive effect varied in magnitude with the intensity of the conditioning stimulus in both A δ -C and C-A δ experiments. Furthermore, intra-segmental interaction was differentially effective for A δ conditioning, (peak amplitude, $p < 0.008$; ANOVA). Our experiments provide the first neurophysiological evidence for a somatotopically distributed, mutually suppressive interaction between cortical responses to preferentially activated A δ and C afferents in humans. This suppressive interaction of cortical responses suggests contrasting and possibly mutually exclusive sensori-motor functions mediated through the A δ and C fiber afferent channels.

Keywords

somatosensory; inhibition; human; evoked potentials; cerebral cortex; pain; temperature; spinothalamic tract

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As a population, spinothalamic neurons receive direct synaptic input from a variety of somatic and visceral afferent fibers. The cutaneous afferent inputs include large diameter myelinated (A β) fibers that are activated only by tactile stimuli and small diameter, finely myelinated (A δ) and unmyelinated (C) fibers innervating receptors responding to innocuous and noxious mechanical and thermal stimulation. Some spinothalamic cells receive converging input from all of these fiber types, but others, such as neurons in laminae 1 and 2 of the spinal dorsal horn, respond only to thermal or noxious stimuli that excite A δ or C fibers (for review, see (Cervero and Iggo, 1980),(Willis, 2004) and (Craig, 2003)).

Some A δ and C fibers respond to tactile stimuli (Traub and Mendell, 1988; Vallbo et al., 1993), but a unique characteristic of both A δ and C fibers is that they innervate somatic thermoreceptors and nociceptors (Burgess and Perl, 1967; Bessou and Perl, 1969; Beitel and Dubner, 1976; Perl, 1984; Traub and Mendell, 1988). Anatomical and physiological studies have consistently demonstrated the convergence of these inputs onto spinothalamic tract neurons in the substantia gelatinosa and deeper laminae of the spinal and medullary dorsal horn (Christensen and Perl, 1970; Chung et al., 1979; Light and Perl, 1979a; Gobel et al., 1981; Cruz et al., 1987; Rethelyi et al., 1989; Yoshimura and Jessell, 1989). Most studies have revealed a predominantly excitatory input from both fiber types with the relatively short duration postsynaptic response to stimulation of A δ fibers preceding the C fiber response (Willis et al., 1974; Menetrey et al., 1977; Chung et al., 1979; Yoshimura and Jessell, 1989); this is consistent with the facilitatory effect of cutaneous thermal stimulation on human nocifensive reflexes (Plaghki et al., 1998).

There is evidence, however, that the interactions between the central processes initiated by A δ and C fiber stimulation are complex, as suggested by the synaptic anatomy of the substantia gelatinosa (Light and Perl, 1979b; Cruz et al., 1987; Rethelyi et al., 1989). Electrophysiological investigations reveal both inhibitory and excitatory postsynaptic responses of nociceptive dorsal horn neurons, including inhibitory interactions mediated through the activation of A δ and C fibers (Christensen and Perl, 1970; Chung et al., 1984a; Chung et al., 1984b; Traub and Mendell, 1988; Yoshimura and Jessell, 1989; Tsuruoka et al., 1990; Schneider and Perl, 1994; Shimizu et al., 1995; Sandkuhler et al., 1997; Liu et al., 1998). Subsequent human neurophysiological and psychophysical studies revealed the well-known suppressive effect of tactile and vibrotactile stimulation on various forms of pain and pain-related evoked responses (Melzack and Wall, 1965; Zoppi et al., 1991; Marchand et al., 1991; Kakigi and Shibasaki, 1992; Akyuz et al., 1995; Svensson et al., 1999; Watanabe et al., 1999; Hoshiyama and Kakigi, 2000; Nahra and Plaghki, 2003) but the opposite interaction has been demonstrated also (Apkarian et al., 1992; Apkarian et al., 1994; Tran et al., 2003). Earlier human neurophysiological studies provided evidence that the activation of A δ -fibers suppresses the cortical response mediated by C-fibers (Bromm and Treede, 1987a; Bromm and Treede, 1987b). Indeed, human cortical responses mediated exclusively by C-fibers can be elicited by only a few methods that selectively activate C-fibers without simultaneously activating A δ -fibers (Magerl et al., 1999) (for review see (Plaghki and Mouraux, 2003)). Early psychophysical studies showed that heat stimulation suppresses A δ fiber-mediated first pain sensation, but this was considered to be caused by suppression of A δ heat nociceptors (Price et al., 1977). However, subsequent psychophysical investigations revealed that innocuous warm stimuli can markedly attenuate heat pain sensation by central mechanisms (Casey et al., 1993) and that homotopic electrocutaneous stimulation of A δ and C fibers can markedly reduce heat and mechanical pain for nearly an hour (Nilsson and Schouenborg, 1999; Nilsson et al., 2003). The cumulative evidence thus suggests that human neurophysiological studies should show inhibitory interactions between supraspinal responses to A δ and C fiber stimulation but this has not been investigated systematically.

To investigate the central, supraspinal interaction of A δ and C fibers and to obtain estimates of the magnitude and site of this interaction, we used recently developed methods for the preferential stimulation of each fiber type (Inui et al., 2002; Granovsky et al., 2005) combined with a paired conditioning stimulation (CS) and test stimulation (TS) paradigm to measure the peak and integrated amplitude of cerebral potentials evoked uniquely by A δ and C fiber stimulation.

Experimental procedures

Subjects

Ten paid healthy volunteers participated in this study. There were two experiments (see below); four subjects participated in both experiments. In experiment 1, the seven subjects (3 males and 4 females) were 24.7 ± 7.8 (mean $\pm$ SD) years old; in experiment 2, the seven subjects (3 males and 4 females) were 24.4 ± 8.0 (mean $\pm$ SD) years old. The local institutional review board of the Ann Arbor Veteran's Affairs Medical Center approved the study protocol. Each subject signed a consent form after receiving a complete explanation of the purpose and design of the study.

Preferential A δ -fiber stimulation

We used electrical epidermal stimulation, similar to the method described previously (Inui et al., 2002). We placed a push-pin needle electrode (cathode) on the dorsum of the left hand between the first and second metacarpal bones. The needle electrode is mounted in the center of a $4 \times 4 \times 3$ cm polyoxymethylene block, which is strapped to the hand so that the needle tip penetrates into the epidermis. A plastic stop device on top of the needle limits epidermal penetration to 0.4 mm. To eliminate any risk for blood-borne infections the stainless steel needle electrodes are steam sterilized between subjects (132°C , 4 min). A dry-gel surface electrode (anode, 20 mm in diameter) is placed on the hand dorsum at around mid-point of the fourth metacarpal bone. We applied constant current square wave pulses of 1 ms duration at intensities just sufficient to evoke a pinprick sensation (pp) at the lowest intensity and at twice that level (2pp) for higher intensities (see below).

Preferential C-fiber stimulation

To activate C fibers preferentially, we used a contact heat evoked potential stimulator (CHEPS) on glabrous skin. (Granovsky et al., 2005) The CHEPS has a thermode with a skin contacting area of 572.5 mm^2 (Medoc Ltd, Ramat Yishai, Israel). The thermode is comprised of an external heating thermofoil (Minco Products, Inc., Minneapolis, MN) covered with a 25 micron layer of thermoconductive plastic (Kapton®). Two thermocouples are embedded 10 microns within this conductive coating, which contacts the skin directly, thus providing an indirect estimate of the skin temperature at the thermode surface. The thermofoil permitted a heating rate of up to 70°C/s (without skin contact) and an underlying thermistor-controlled Peltier device maintained baseline temperature and allowed a heating and cooling rate of $\sim 40^\circ\text{C/s}$ in contact with the skin. Cooling began immediately following attainment of the target heat pulse temperature, which was set by the investigator using software provided by the manufacturer. In this study, the baseline temperature was 35°C and the target temperature was 37°C and 50°C . The heat stimulus was a rapidly increasing and decaying heat wave pulse with a full-width-half-maximum duration of approximately 350 ms.

Evoked potential (EP) recordings

We acquired data with the Neuroscan 4.3 system (Compumedics USA, El Paso, TX, USA) running continuously during each recording session; we used this system to store and analyze data off-line. The exploring electrode was placed at Cz according to the 10–20 International

System, and was referred to linked earlobes (A1 + A2). The ground electrode was placed on the forehead between Fpz and Fz. Impedance was maintained below 5k Ω . The EP was recorded at a filter frequency of 0.1–100 Hz and a sampling rate of 500 Hz. The recording epoch spanned 2100 ms including 100 ms pre- and 2000 ms post-stimulus. Time zero was indicated by the onset of the CS, which was signaled to the recording system at the onset of the electrical (A δ) stimulation and at the offset of a TTL pulse initiating the heat (C) stimulation. Electrooculography was simultaneously recorded for artifact rejection.

Experimental design

We used the conditioning stimulus (CS) - test stimulus (TS) paradigm in this study. There were two experiments, one with A δ as CS and C as TS and another one with the conditions reversed. The inter-stimulus intervals of 200 ms with A δ as CS and 1000 ms with C as CS were based on a pilot study showing that these interstimulus intervals assured that the CS potentials would arrive at the cortex at least 500 ms before the TS signals. This cortical response interval permitted accurate measurements of the response to the TS without interference from the response evoked by the CS and avoided attentional factors (see Discussion).

In both experiments, the intradermal electrical A δ stimulation was applied at the dorsum of the left hand; this placement was maintained throughout the experiment to maintain a reliable sensation produced at that electrode position. The C fiber stimulation was applied by moving the contact thermode to the left thenar, hypothenar, and right thenar eminence, corresponding to intra-segmental, inter-segmental and contralateral C fiber stimulation relative to A δ stimulation. Thus, we tested 3 interactions for each experiment: intrasegmental (Intra), intersegmental (Inter) and contralateral (Con). The intensity of the conditioning and testing stimuli varied depending on the interaction under study (see below). To avoid changes in peripheral sensitivity, we used stimulus intensities that were just sufficient to evoke cerebral potentials consistently.

Table 1 shows an example of the data acquisition schedule for a typical subject in experiment 1 and experiment 2. We delivered 80 stimulation trials for each conditioning configuration (A δ -C and C-A δ), 40 during Intra and 20 each during Inter and Con testing. To avoid habituation and subject fatigue in both experiment 1 and experiment 2, we examined intrasegmental (Intra) conditioning with two intensities of A δ stimulation (pp and 2pp) in one session on one day (20 + 20 stimuli), and Inter and Con conditions with A δ stimulation at pp or 2pp intensity in another session (40 stimuli) on a different day. Subjects had a 3–5 min rest between runs of 20 stimulation trials. Sequences of sessions and conditions within each session were pseudorandom and counter-balanced among subjects. Subjects sat in an armchair in a quiet room with an ambient temperature of approximately 24°C throughout each experimental session.

Experiment 1: A δ -fiber CS and C-fiber TS (A δ -C)

To detect the effect of A δ conditioning stimulus intensity, we used two intensities of A δ stimulation, pp and 2pp, when C stimuli were applied to the thenar (intra-segmentally: Intra-pp and Intra-2pp). In anticipation of weaker extrasegmental effects, and to minimize subject fatigue, we used only an intensity of 2pp for A δ stimulation when we applied C stimulation inter-segmentally (hypothenar, Inter) or contralaterally (Con). The temperature of C stimulation was 50°C for all conditions because this stimulus produced potentials that were sufficiently large and consistent for accurate measurements.

In each run of 20 stimulus trials, ten trials were C-only as control without A δ stimulation and ten trials were with paired stimuli (CS-TS of A δ -C). The A δ -CS was applied 200 ms before C-TS. The C-only and A δ -C trials were given randomly, and the inter-trial intervals were

randomized between 10 and 15 s. The mean pp stimulus intensity of A δ stimulation was 0.20 ± 0.09 mA (mean $\pm$ SD). Thus, all electrical stimuli were well below the very high electrical threshold for the excitation of C fibers by direct nerve root stimulation (Ikeda et al., 2000; Ling et al., 2003) or by intracutaneous stimulation in rats (Falinower et al., 1994) or humans (Schmidt et al., 2002); the stimulation parameters were also well outside the range that has been used to evoke C-fiber-mediated itch (Ikoma et al., 2005).

Experiment 2: C-fiber CS and A δ -fiber TS (C-A δ)

To detect the effect of the intensity of both conditioning and testing stimuli, we used two intensities of C-fiber conditioning stimulation (37 and 50°C) and two intensities of A δ -fiber stimulation (pp and 2pp) during the Intra interaction condition. This design allowed us to test the intensity effects of the conditioning stimulation and avoid the extra stimuli that would be required if heat stimuli were applied alone as a control. In anticipation of weaker conditioning effects, we used only pp A δ test stimulation for Inter and Con interactions; these potentials were sufficiently large and consistent for accurate measurements. Subjects received two runs of 20 trials each for each condition. In each run, ten trials were C37-A δ with the low target temperature at 37°C for comparison with ten trials of paired stimulation at 50°C (C50-A δ). The C-CS was applied 1000 ms before A δ -TS. The C37-A δ and C50-A δ trials were given randomly, and the inter-trial intervals were randomized between 10 and 15 s. The mean pp stimulus intensity was 0.18 ± 0.06 mA (mean $\pm$ SD).

Verbal Sensation Rating (SR)

To assure attention to the stimuli, there was a verbal warning signal 3–5 s before each trial. Subjects were asked to rate the perceived intensity of CS and TS stimuli 3 s after the trial. For both the electrical and heat stimuli, the ratings were based on a 0–10 scale, the extremes of which were “no sensation” at 0 and “intolerable pain” at 10. A level of 4 was the designated pain threshold. For example, if the subject said “4, 3” that meant he felt two stimuli, and the rating of CS was 4 and TS was 3.

Data analysis

The two runs in each condition confirmed the reproducibility of the responses. For quantitative analysis, we averaged twenty artifact-free trials of 2 runs in each condition. The peak latency and amplitude, and integrated amplitude (total microvolt values for the data points) of EP were determined from these averaged waveforms. First, to determine the effect of conditioning (TS only vs. CS-TS), site and intensity we performed two two-way repeated measures analysis of variance (ANOVA) of the peak and integrated amplitude and the sensory rating (SR) in each experiment. In experiment 1 (A δ -C) the factors of the two two-way ANOVAs were conditioning and TS site (intra, inter, contra), and conditioning and CS intensity (pp, 2pp). In experiment 2 (C-A δ) the factors were conditioning intensity (C37 vs. C50) and CS site (intra, inter, contra) and conditioning intensity and TS intensity (pp vs. 2pp). To determine the effect of conditioning (TS only vs. CS-TS) in experiment 2, we used a two-way ANOVA (without repeated measures) to compare group means of the amplitudes of the unconditioned A δ responses in experiment 1 with those of the conditioned A δ responses in experiment 2. Second, to investigate further the attenuating effect across conditions, we expressed the peak and integrated amplitude values as a ratio, a standardized value, which was obtained by dividing each test value by the control value for each individual in each experiment in the same condition. We performed a one-way analysis of variance of these standardized values to determine the effect of conditioning across conditions and applied the Tukey HSD test for post-hoc comparisons. In all cases, *P*-values of less than 0.05 were considered significant. We used SPSS 11.5 and 14.0 software for statistical analyses.

Results

A δ and C evoked potentials

In experiment 1, we identified the positive phase (P2) of A δ - and C-mediated responses in all recordings except for P2 of the C response in the Intra-2pp condition in one subject (Table 2, footnote c). We identified the negative N2 potentials of C-only responses in only 8 out of 28 recordings (4 conditions $\times$ 7 subjects) and only one subject showed N2 potentials in all four conditions. Because the negative N2 potentials were not consistent, we analyzed only the P2 component of C responses. In experiment 2, we identified the P2 potentials of C responses in all C50-A δ tests, but in only 9 out of 28 recordings (4 conditions $\times$ 7 subjects) during C37-A δ testing. The P2 potentials of A δ responses were defined in all recordings except for two recordings of the Intra-pp condition (Table 2, footnote d). The negative N2 potentials of A δ responses were recognized in only 11 out of 28 recordings (4 conditions $\times$ 7 subjects) of C37-A δ , and only one subject showed N2 potentials in all four conditions. Because the negative N2 potentials were not consistent, we analyzed only the P2 component of A δ responses.

We estimated response latencies of both A δ and C mediated responses in the same subjects and under the same conditions when the electrical stimulator was used to trigger the Neuroscan recorder. The mean latency of the peak P2 component of the unconditioned A δ mediated response (the CS) ranged from 289.9 (20.6 s.d.) ms during 2pp stimulation to 308.3 (19.1 s.d.) during pp stimulation. With the electrical CS stimulator off, we estimated the mean latency of the peak P2 component of the unconditioned C mediated response. We used the C mediated response to 50°C for latency estimation because it was the most reliable (see above) and found that the mean latencies ranged from 632.9 (52.2 s.d.) to 688.6 (134.1 s.d.) ms. The A δ mediated response latency is consistent with the average latency of 302.8 ms estimated by (Inui et al 2002) for epidermal electrical stimulation of the hand. The C mediated response is slightly longer than the average C mediated response estimated by Granovsky et al (2005) (611 ms) from CHEP thenar stimulation; however, group differences in arm length are likely to contribute to these latency differences because the participants in the Granovsky study were selected to maximize the range of arm lengths.

The means and standard deviations of the peak and integrated amplitudes of P2 of A δ (A δ -P2) and P2 of C (C-P2) responses, and their mean standardized values in experiment 1 (A δ -C) and 2 (C-A δ) are summarized in Table 2.

Effect of A δ on C cerebral responses

Under all conditioning configurations, the electrical stimulation of A δ fibers attenuates the response to the stimulation of heat-sensitive C fibers (Fig. 1 and Fig. 2). Table 3 gives the statistical summary of the two-way ANOVA analyses, showing a significant main effect of conditioning across site ($p < 0.0001$) and intensity ($p < 0.0001$) on peak evoked potential amplitude; the main effects are confirmed for the integrated amplitude (not shown; intensity, $p < 0.004$; site, $p < 0.006$). There is a strong interaction of conditioning and site ($p = 0.008$) with evidence for a greater effect of Intra and Contra conditioning ($p < 0.001$) compared to Inter conditioning ($p = 0.045$). Likewise, there is a strong interaction of conditioning and stimulus intensity ($p < 0.0001$) with evidence for a greater effect of the stronger conditioning stimulus. Figure 2 is a graphical presentation of these results.

Normalized values of the peak amplitudes were < 1 in all conditions and subjects (Fig. 3). The ANOVA applied to these values showed that the peak amplitudes of C-P2 of A δ -C are significantly smaller than those of C-P2 of C-only in all conditions ($F_{4,28} = 31.9$; $p < 0.0001$). There is an effect of stimulus intensity with Intra 2pp being more effective than Intra pp ($p < 0.0001$) and, on post-hoc testing (Tukey HSD) of stimulus site with Intra 2pp being more

effective than either Inter 2pp ($p = 0.014$) or Contra 2pp ($p = 0.012$) and with no difference between the Inter and Contra conditioning ($p = 1$).

A two-way repeated measures ANOVA did not reveal any effect of conditioning on the latency of C-TS responses between A δ -C and C-only in Experiment 1.

Effect of C on A δ cerebral responses

We observed a significant attenuating effect of C-CS on A δ -TS (Figure 4, Figure 5 and Table 4). Table 4 gives the statistical summary of the two-way ANOVA analyses, showing a significant main effect of both C37 and C50 conditioning across sites ($p = 0.003$) and the greater effect of C50 compared to C37 conditioning (C37 vs C50; $p = 0.019$) on peak evoked potential amplitude; the main effects are confirmed for the integrated amplitude (not shown; intensity, $p = 0.016$; across sites, $p = 0.006$). As expected, the TS response to the 2pp stimulus exceeded that to the pp stimulus, but this did not apparently affect conditioning. There is no interaction of site with stimulus intensity. A separate analysis (2 way ANOVA without repeated measures) shows that both the C37 and C50 conditioning stimuli (CS) are effective at both intensities of test stimulus intensity (pp, 2pp) when compared with the unconditioned A δ -mediated responses of experiment 1 ($p < 0.0001$; only pp intensity used for Inter- and Contra- test stimuli (TS) in experiment 2).

Figure 5 is a graphical presentation of these results.

Because we used only pp stimulus intensity for the TS during Inter and Contra conditioning in this experiment, we wished to test independently the effect of C37 Intra conditioning when both pp and 2pp TS were used. We used a 2-way ANOVA to compare the group means of the amplitudes of the unconditioned intrasegmental A δ responses in experiment 1 with those of the conditioned intrasegmental A δ responses only during intrasegmental conditioning in experiment 2 when both pp and 2pp TS intensities were used. The results (Fig. 6) show a significant main effect of C37 conditioning ($F_1 = 15.9$; $p = 0.001$) on the peak amplitudes of the TS A δ responses to both pp and 2pp stimulus intensities, no interaction of conditioning with the effect of TS on evoked potential amplitude ($F_1 = 0.093$; $p = 0.764$), and the expected main effect of stimulus intensity on TS amplitude ($F_1 = 4.9$; $p = 0.039$). Inspection of the data suggests a trend for a weaker conditioning effect on the stronger TS (Fig.6).

Normalized values of the peak and integrated amplitude were < 1 in all conditions and all subjects except 1 (Fig. 7). The ANOVA applied to these values showed that the peak amplitudes of A δ -P2 of C50-A δ are significantly smaller than those of C37-A δ in all conditions ($F_{4,28} = 5.50$; $p = 0.002$). There is no effect of test stimulus intensity or of stimulus site, Intra pp being no more effective than Inter or Contra pp.

A two-way repeated measures ANOVA did not reveal any effect of conditioning on the latency of A δ -TS responses between C50-A δ and C37-A δ in Experiment 2.

Sensation Rating (SR) score

In both experiments, subjects experienced a pin prick sensation with A δ stimulation and a heat sensation with C stimulation. On average, none of the stimuli was rated as painful. The mean SR of the CS was 3.1 ± 0.55 sd for the A δ fiber stimulus and 2.9 ± 0.61 sd for the C fiber stimulus. In experiment 1, there was a trend for the C-mediated response to be perceived as more intense during intrasegmental A δ conditioning (post hoc t test, $t_6 = -3.2$; Bonferroni corrected $p = 0.06$). In experiment 2, C-mediated conditioning had no significant effect on the rating of the A δ test stimulus at either location or intensity. The A δ test stimulus was, however, rated as stronger at 2pp than at pp stimulus intensities ($F_{1,6} = 9.8$; $p = 0.02$).

Discussion

Our data provide neurophysiological evidence for a mutually suppressive interaction between stimuli that preferentially excite A δ and C afferents in humans. The neurophysiological characteristics of the cortical responses to these stimuli were nearly identical to those reported by Inui et al (Inui et al., 2002) and by Granovsky et al (Granovsky et al., 2005) for A δ and C fiber mediated evoked potentials. The cortical response to preferential A δ or C fiber stimulation was suppressed whenever either cortical response preceded the other. The A δ and C suppressive conditioning occurred when applied at all conditioning sites and, for A δ conditioning, intra-segmental interaction was differentially effective as shown in the examination of the normalized responses. The suppressive effect also varied in magnitude with the intensity of a conditioning stimulus in both A δ -C and C-A δ experiments. In the C- A δ experiment, we showed that the 50°C, compared with the 37°C stimulus, was differentially effective in attenuating the A δ -mediated response. We also showed, by a group comparison of the unconditioned and conditioned A δ responses, that the 37°C conditioning stimulus attenuated the A δ response.

We could not detect any significant difference of SR score and peak latency in either experiment. The lack of perceived intensity difference may be because the brevity of each stimulus did not provide the temporal summation necessary for accurate intensity estimation (Torebjork et al., 1984; Magerl et al., 1999); in addition, the short intervals between the CS and TS may have impaired the ability of our subjects to discriminate clearly between them and to evaluate intensity differences easily. Finally, the evoked potentials we recorded at Cz are probably generated within or near the anterior cingulate gyrus (Lenz et al., 1998) and their amplitudes may not be specifically and exclusively related to perceived stimulus intensity.

Selectivity of the stimulation

It is never possible to be certain that cutaneous stimuli completely exclude one or another fiber type. Tactile stimuli, for example may activate both C and A β fibers (Vallbo et al., 1999). However, our methods provided a strongly predominant, if not absolutely exclusive, stimulation of one particular fiber type. We used an intradermal electrode method that evokes a cerebral potential and waveform that is morphologically similar to the well-known LEP and that is mediated by A δ fibers (Inui et al., 2002). We found no electrophysiological evidence, using high gain recordings, for the activation of A β fibers, and our subjects clearly perceived the pin prick sensation that is strongly associated with the A δ -mediated LEP. For the C fiber stimulation, we used a method that has been shown to be C fiber-mediated by conduction velocity measurements and that evokes only the sensation of warmth without a tactile or pin prick component when applied to glabrous skin (Granovsky et al., 2005). The shorter latency of this response, compared to C fiber-mediated potentials evoked by infrared laser stimulation (Tran et al., 2001; Opsommer et al., 2001), is probably due to several factors, including a longer stimulus duration (approximately 10 to 300 times longer) and a much larger stimulation area (30 to 3000 times greater). We could not detect electrophysiological evidence for the activation of A δ fibers with this stimulus. Indeed, when this contact heat stimulus is applied to hairy skin at temperatures sufficient to produce a slightly painful pin prick sensation, only waveforms consistent with A δ fiber stimulation appear. Therefore, for C fiber activation, we intentionally used painless warm stimuli at temperatures well below those necessary to stimulate the heat nociceptors innervated by A δ fibers (Treede et al., 1995).

Consideration of attentional factors

The effects of attention and expectation are difficult to eliminate completely in these experiments. However, in the A δ -C experiments, the attenuation effect of CS on TS, although evoked from all conditioning sites, was significantly greater during intrasegmental A δ -C

conditioning; this result would not be expected if attentional or habituation factors were responsible for our results. Stimulus expectation might contribute to the reduced amplitude of A δ mediated laser-evoked potentials (LEP) as shown in the conditioning-test paradigm of Truini and colleagues where the paired laser stimuli were delivered repetitively at fixed intervals (Truini et al., 2004). However, these investigators did not measure expectation and did not require the subjects to attend to and rate each stimulus. In our experiment, subjects were asked to rate each stimulus, an attentional factor that should have increased, or at least attenuated the decrease of, the LEP (for review, see (Lorenz and Garcia-Larrea, 2003)). This conjecture is supported by the results of Mouraux and colleagues (Mouraux et al., 2004) who required stimulus ratings of each of two consecutive laser stimuli with randomly varying stimulus onset and interstimulus intervals and found that the late-LEP response to the second stimulus was not altered. It is unlikely also that the response attenuation is due primarily to a shift of attention toward the CS and from the TS. First, in the A δ -C experiment, intradermatomal conditioning uniquely enhanced the attenuation effect. If attention had played a role here, the attenuation effect should have been at least as strong during contralateral conditioning because the subjects' attention would have been directed toward the contralateral side (Legrain et al., 2002; Lorenz and Garcia-Larrea, 2003). Secondly, Ward and colleagues (Ward et al., 1996) have presented evidence that visual attentional shifts to a second stimulus do not occur earlier than 500 ms after the first stimulus. In our study, the signals of the CS were designed to reach the cortex no earlier than 500 ms before that of the TS. Assuming that somatosensory attentional shifts occur on average no earlier than those in the visual system, the weight of the evidence presented here favors the interpretation that the attenuation effect found in the present study is not due to expectation effects or to shifts of attention from the TS to the CS but rather results from the physiological interaction of signals mediated by A δ and C fibers. Although we did not find a differential effect of intra-segmental conditioning in the C-A δ experiments, the 500 ms interstimulus interval was the same as in the A δ -C experiment and thus favors a physiological interaction mechanism rather than purely attentional or related cognitive effects.

Intra- and extrasegmental components of the interaction

Our neurophysiological data suggests an intrasegmental component for the A δ -C interaction, which is consistent with a possible spinal site of interaction. However, this somatotopic effect could also be mediated through somatotopically organized ascending or descending processes at all supraspinal levels. In addition, the extrasegmental and contralateral effects of conditioning in both the A δ -C and C-A δ experiments suggest that the interaction must also be mediated through a widely distributed, non-somatotopic mechanism. Overall, the interpretation favors an interaction mediated through both somatotopic and nonsomatotopic mechanisms that receive convergent input from A δ and C fibers.

The interaction of A δ and C signals is intensity dependent

The suppressive interaction varies in magnitude with the intensity of a conditioning stimulus in both A δ -C (pp vs. 2pp) and C-A δ (37°C vs. 50°C) experiments. The A δ -C interaction may explain why, in previous experiments, painful laser stimulation could evoke only the late-LEP A δ response although A δ and C fibers were probably simultaneously activated. To elicit an ultralate-LEP or C response, many methods of selective activation of C fiber have been proposed (for review see (Plaghki and Mouraux, 2003)). All of these methods are designed to avoid concomitant activation of A δ fibers. Although waveforms consistent with an ultralate (C-mediated) LEP have been recorded in the immediate wake of a late (A δ -mediated) LEP, (Magerl et al., 1999) a fiber-specific origin of the suspected ultralate response could not be established; nor could an incomplete suppressive effect of the preceding A δ response be ruled out. Indeed, Towell and colleagues (Towell et al., 1996) noticed that the ultra-late LEPs could be recorded most frequently when the stimulus intensity was low and the late LEPs less well

identified. These observations support our finding that the mutually suppressive interaction depends on the magnitude of the conditioning stimulus.

Although the stimuli we used were not painful, our results are consistent with psychophysical evidence that innocuous warm stimuli can attenuate heat pain sensation (Casey et al., 1993) and that electrocutaneous stimulation of A δ and C fibers can strongly attenuate perceived heat and mechanical pain (Nilsson and Schouenborg, 1999; Nilsson et al., 2003). However, as in the A δ -C experiment, the magnitude of the C-A δ suppressive interaction depends on the intensity of the CS. Thus, the 50°C stimulus was more effective than the 37°C stimulus in suppressing the A δ -TS response. Taken together, the results show that the conditioned cerebral responses to the preferential stimulation of either A δ or C fibers depend on the relative input activity of the A δ and C signals.

Mechanisms mediating the suppressive interaction

Based on a time-frequency analysis of electroencephalographic epochs, Mouraux et al (Mouraux et al., 2003) hypothesized that the brain processes first engaged by A δ fibers could not be reactivated by a subsequent C fiber volley. However, as noted above, Mouraux and colleagues also found that the second of two A δ LEP responses was not altered at interstimulus intervals ranging between 280 and 2100 ms (Mouraux et al., 2004). If, according to the latter results, similar inputs do not interact within the time interval we tested, then the interaction we observed between two distinctly different fiber types must be mediated through a neuronal mechanism that receives converging input from A δ and C fibers and differentiates between them to suppress responses evoked from a different, delayed afferent source. We did not, however, examine the effect of predominant C fiber conditioning on the cortical response to the same C fiber stimulus. It is therefore possible that C fiber conditioning produces a more generalized attenuating effect on the amplitude of cortical somatosensory responses¹. In addition, we did not investigate the effect of interactions with tactile stimuli, so our experiments do not provide information about mechanisms related to interactions with A β -mediated responses.

Physiological and clinical significance

Our results demonstrate an early inhibitory interaction between the two fiber types that provide the major inputs to spinothalamic pathways mediating pain and temperature sensations¹. Although these brief stimuli were not painful, their suppressive interactions may nonetheless be relevant for pain-related sensations and behaviors because heat pain requires the neural substrates for painless heat and cold (Defrin et al., 2002) and the just-supraliminal stimulation of heat nociceptors evokes painless warmth (Green and Cruz, 1998). In this broader context, the suppressive cortical interaction between A δ and C fiber pathways may reflect a) the well-established suppressive interaction of noxious stimuli (Bouhassira et al., 1993; Reinert et al., 2000) and b) the activation of contrasting and perhaps mutually exclusive behaviors: an A δ -mediated channel for rapid escape from threat and a slow C-mediated channel that promotes avoidance and the protection of injured tissue.

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¹While this report was under review, Truini and colleagues published a report showing that C fiber conditioning attenuates the amplitude of both A δ and C-mediated responses evoked by spatially adjacent laser stimuli. Their results and ours are consistent with a suppressive interaction that may in part reflect a relative refractoriness of cortical mechanisms mediating orienting responses. Our study additionally demonstrates extrasegmentally and, specifically for A δ conditioning only, intrasegmentally mediated components of this interaction (Truini et al., 2007).

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Glossary

Abbreviations

ANOVA

analysis of variance

CHEPS

contact heat evoked potential stimulator

Con

contralateral

CS

conditioning stimulus

EP

evoked potential

Inter

intersegmental

Intra

intrasegmental

LEP

laser evoked potential

N2

second negative wave

P2

second positive wave

pp

pin prick intensity

2pp

twice pin prick intensity

SD

	standard deviation
SE	standard error of the mean
SR	sensory rating
TS	testing stimulus

Fig. 1.

Waveforms of A δ -C experiment in subject 1. Paired records show P2 waves (arrows) of evoked responses to unconditioned testing C (C-only, upper records) and conditioning A δ fiber stimulation (A δ -C, lower records). All four conditioning patterns are shown from top to bottom: intrasegmental at 2x pin prick threshold (Intra-2pp); intrasegmental at pin prick threshold (Intra-pp); intersegmental at 2x pin prick threshold (Inter-2pp); and contralateral at 2x pin prick threshold (Con-2pp). The peak and integrated amplitudes of C-P2 were smaller during A δ -C conditioning than during the C-only condition.

Fig. 2.

Graphical summary of the analysis of the effect of A δ conditioning on the mean ($\pm$ s.e.m) C-mediated cortical response amplitude (C-P2). The C-mediated response (TS) is attenuated by the conditioning stimulus at all sites (left graph) but the intrasegmental (Intra) and contralateral (Contra) sites are most effective (see Table 3). The conditioning stimulus (CS) is effective at both intensities (2pp and pp) but most effective at the 2pp intensity (see Table 3).

Fig. 3.

Suppressive effect of A δ conditioning stimulation on C-mediated cerebral responses to 50°C stimulation across each of 4 conditions for each individual. Group mean ($\pm$ s.e.m.) shown as solid line connecting the group means. The electronically averaged response amplitude obtained in each condition is standardized (normalized) by dividing the amplitude of the conditioned response by the amplitude of the control response for each individual. Therefore, there is only one value for each individual in each condition and no estimate of intra-individual variance; inter-individual variance estimates are shown in Table 2. Standardized values of peak amplitude were < 1 in all conditions for all subjects ($p < 0.0001$). There is an effect of stimulus intensity with Intra 2pp being more effective than Intra pp (**; $p < 0.0001$) and of stimulus site with Intra 2pp being more effective than either Inter 2pp (*; $p = 0.014$) or Contra 2pp (*; $p = 0.012$).

Fig. 4.

Waveforms of C-A δ experiment in subject 6. Same general format as Figure 1 except that the C conditioning stimulus is shown in each record and was applied at 37°C (upper records) and at 50°C (lower record) in each condition. In addition, the intensity of the A δ test stimulus was applied at both 2x pin prick (2pp) and pin prick (pp) intensities during intrasegmental (Intra) conditioning but only at pp intensities during Inter- and Con- conditioning. The peak and integrated amplitudes of the A δ -P2 response was smaller during C conditioning at 50°C than during C conditioning at 37°C in all conditions.

Fig. 5.

Graphical summary of the analysis of the effect of C conditioning on the mean ($\pm$ s.e.m.) A δ -mediated cortical response amplitude (A δ -P2; see Table 4). The A δ -mediated response is more attenuated by the C50 than by the C37 conditioning stimulus (left graph; **, $p = 0.003$) but there is no difference across sites. The C50 stimulus intensity is more effective than the C37 stimulus (right graph; *, $p = 0.02$) but there is no difference across test stimulus intensities (pp, 2pp). A separate analysis (ANOVA without repeated measures) shows that the C37 and C50 conditioning stimuli (CS) are effective at both intensities of test stimulus intensity (pp, 2pp) when compared with the unconditioned A δ -mediated responses of experiment 1 ($p < 0.0001$; only pp intensity used for Inter- and Contra- test stimuli (TS) in experiment 2).

Fig. 6.

Suppressive effect of intrasegmental C conditioning (37°C stimulation) on Aδ cerebral responses to test stimulation (TS) at pp and 2pp intensities. Average ($\pm$ s.e.m.) unconditioned peak amplitude of Aδ evoked potential (left pair of bars) compared with average conditioned Aδ evoked potential amplitude (right pair of bars). Hatched bars from 6 participants in experiment 1 (Aδ-C); stippled bars from 5 participants in experiment 2 (C-Aδ). The 37°C stimulus attenuated the Aδ responses to both 2pp (*, $p < 0.025$) and pp (**, $p < 0.008$) stimulation and conditioning did not alter the effect of TS stimulus intensity on evoked potential amplitude ($p = 0.764$; 2-way ANOVA).

Adelta P2: C₅₀ vs C₃₇ CONDITIONING

Fig. 7.

Suppressive effect of C₅₀ vs C₃₇ conditioning stimulation on normalized A δ -mediated cerebral responses to stimulation across each of 4 conditions for each individual. Group mean ($\pm$ s.e.m.) shown as solid line. Format is the same as for Figure 3. Normalized values of peak amplitude were < 1 in all conditions for all subjects but one ($F_{4,28} = 5.5$; $p = 0.002$). There is no effect of stimulus site or of test stimulus intensity.

TABLE 1
SAMPLE PROTOCOL SCHEDULE FOR A PARTICIPANT

Aδ - C				C - Aδ			
day/run ¹	#stim ²	Conditioning	Test	day/run ¹	#stim ²	Conditioning	Test
1/1	20	intra pp	C50	3/1	20	C50	intra pp
	10	0 (control)	C50		20	C37	intra pp
1/2	20	intra 2pp	C50	3/2	20	C50	intra 2pp
	10	0 (control)	C50		20	C37	intra 2pp
2/1	20	inter 2pp	C50	4/1	20	C50	inter pp
	10	0 (control)	C50		20	C37	inter pp
2/2	20	contra 2pp	C50	4/2	20	C50	contra pp
	10	0 (control)	C50		20	C37	contra pp

¹ Each run was repeated once to confirm reproducibility of responses.

² Includes conditioning and test stimuli.

Table 2

Mean and standard deviation of the peak and integrated amplitudes of P2 of A δ (A δ -P2) and P2 of C (C-P2) responses, and their mean standardized values in experiment 1 (A δ -C) and 2 (C-A δ).

Aδ-C^a	Intra-2pp	Intra-pp	Inter-2pp	Con-2pp
Peak amplitude (μ V)				
A δ -P2 peak of A δ -C	33.1 $\pm$ 14.5	25.2 $\pm$ 10.3	33.4 $\pm$ 9.0	28.2 $\pm$ 7.9
C-P2 peak of A δ -C	10.1 $\pm$ 4.6 ^c	15.4 $\pm$ 6.6	9.9 $\pm$ 4.9	9.3 $\pm$ 4.9
C-P2 peak of C only	28.8 $\pm$ 8.4	21.8 $\pm$ 8.5	20.8 $\pm$ 12.9	17.4 $\pm$ 4.6
Mean stand. values of C-P2	0.27 $\pm$ 0.18	0.71 $\pm$ 0.08	0.52 $\pm$ 0.11	0.51 $\pm$ 0.15
Integrated amplitude (μ V)				
C-P2 of A δ -C	298 $\pm$ 242 ^c	500 $\pm$ 191	517 $\pm$ 297	482 $\pm$ 192
C-P2 of C only	1258 $\pm$ 565	1194 $\pm$ 495	1244 $\pm$ 726	1220 $\pm$ 489
Mean stand. values of C-P2	0.22 $\pm$ 0.19	0.47 $\pm$ 0.21	0.45 $\pm$ 0.16	0.41 $\pm$ 0.16
C-Aδ^b	Intra-2pp	Intra-pp	Inter-pp	Con-pp
Peak amplitude (μ V)				
C-P2 37C peak of C-A δ	14.3 $\pm$ 7.3	14.3 $\pm$ 4.6	12.6 $\pm$ 2.5	8.5 $\pm$ 1.5
C-P2 50C peak of C-A δ	17.9 $\pm$ 8.7	18.0 $\pm$ 8.1	15.5 $\pm$ 5.9	13.4 $\pm$ 4.3
A δ -P2 peak of 37C-A δ	16.4 $\pm$ 9.4	11.2 $\pm$ 6.9 ^d	15.1 $\pm$ 6.9	14.0 $\pm$ 6.8
A δ -P2 peak of 50C-A δ	11.3 $\pm$ 8.4	6.9 $\pm$ 5.8 ^d	9.0 $\pm$ 7.5	8.0 $\pm$ 4.7
Mean stand. values of A δ -P2	0.66 $\pm$ 0.19	0.42 $\pm$ 0.34	0.56 $\pm$ 0.34	0.57 $\pm$ 0.13
Integrated amplitude (μ V)				
A δ -P2 of 37C-A δ	827 $\pm$ 414	535 $\pm$ 151 ^d	1136 $\pm$ 511	870 $\pm$ 412
A δ -P2 of 50C-A δ	491 $\pm$ 358	288 $\pm$ 177 ^d	373 $\pm$ 248	327 $\pm$ 241
Mean stand. values of A δ -P2	0.61 $\pm$ 0.24	0.35 $\pm$ 0.35	0.30 $\pm$ 0.29	0.43 $\pm$ 0.26

^aCS-TS interval adjusted from 200 ms

^badjusted from 1000 ms

^cfrom 6 subjects

^dfrom 5 subjects.

TABLE 3

Statistical summary of A δ -C experiment.

	<i>dF</i>	Effect on P2 peak amplitude of C <i>F, t</i>	<i>p</i>
Main effects - TS site			
Conditioning (C only vs. A δ -C)	1,5	89.4	< 0.0001
Site (intra, inter, extra)	2,10	2.6	0.153
Site (intra, inter, extra) ²	1.4, 6.9	2.6	0.153
Interactions			
Conditioning (C only vs. A δ -C) $\times$ Site	2,10	8.2	0.008
Conditioning (C only vs. A δ -C) $\times$ Site ²	1.6, 8.1	8.2	0.014
Post hoc - Site¹			
Conditioning intra	5	11.6	< 0.001
Conditioning inter	6	3.4	0.045
Conditioning contra	6	11.1	< 0.001
Main effects - CS intensity			
Conditioning (C only vs. A δ -C)	1,5	154.6	< 0.0001
CS Intensity (2pp, pp)	1,5	0.002	0.97
Interactions			
Conditioning(C only vs. A δ -C) $\times$ CS Intensity	1,5	71.8	< 0.0001
Post hoc - Intensity¹			
Condition 2pp	5	11.6	< 0.001
Condition pp	6	6.0	< 0.005

¹ Bonferroni corrected² Greenhouse-Geisser corrected*dF* - degrees of freedom, *F* - F statistic, *t* - t statistic, *p* - p-value (bold if < 0.05)

TABLE 4Statistical summary of C-A δ experiment

	<i>dF</i>	Effect on P2 peak amplitude of A δ <i>F</i>	<i>p</i>
Main effects - TS site			
CS Intensity (C37 vs. C50)	1,4	38.9	0.003
Site (intra, inter, contra)	2,8	1.0	0.39
Site (intra, inter, contra) ²	1.2, 4.7	1.0	0.39
Interactions			
CS Intensity (C37 vs. C50) $\times$ Site	2,8	0.3	0.65
CS Intensity (C37 vs. C50) $\times$ Site ²	1.1, 4.6	0.3	0.65
Main effects - TS and CS intensity			
CS Intensity (C37 vs. C50)	1,4	14.5	0.019
TS Intensity (2pp, pp)	1,4	14.4	0.019
Conditioning (A δ only vs. C37- A δ) ¹	1.24	16.7	<0.0001
Interactions			
CS intensity $\times$ TS intensity	1,4	0.1	0.79
Conditioning (A δ only vs. C37- A δ) $\times$ TS intensity	1.24	0.0	0.93

¹ Not repeated measures because of different subjects in experiment 1 & experiment 2² Greenhouse-Geisser corrected*dF* - degrees of freedom, *F* - F statistic, *t* - t statistic, *p* - p-value (bold if < 0.05)