Responses of Spinothalamic Lamina I Neurons to Maintained Noxious Mechanical Stimulation in the Cat

D. ANDREW AND A. D. CRAIG
Atkinson Pain Research Laboratory, Division of Neurosurgery, Barrow Neurological Institute, Phoenix, Arizona 85013
Received 13 July 2001; accepted in final form 6 December 2001

Andrew, D. and A. D. Craig. Responses of spinothalamic lamina I neurons to maintained noxious mechanical stimulation in the cat. J Neurophysiol 87: 1889–1901, 2002; 10.1152/jn.00577.2001. Noxious mechanical stimuli that are maintained for minutes produce a continuous sensation of pain in humans that augments during the stimulus. It has recently been shown with systematic force-controlled stimuli that, while all mechanically responsive nociceptors adapt to these stimuli, the basis for such pain can be ascribed to A-fiber rather than C-fiber nociceptors, based on distinctions in their respective response profiles and stimulus-response functions. The present experiments investigated whether similar distinctions could be made in subsets of nociceptive lamina I spinothalamic tract (STT) neurons using similar maintained stimuli. Twenty-eight lamina I STT neurons in the lumbosacral dorsal horn of barbiturate-anesthetized cats were tested with noxious mechanical stimuli applied with a probe of 0.1 mm$^2$ contact area at forces of 25, 50, and 100 g for 2 min. The neurons were classified as nociceptive-specific (NS, $n = 14$) or polymodal nociceptive (HPC, $n = 14$) based on their responses to quantitative thermal stimuli. The NS neurons had greater responses and showed less adaptation than the HPC neurons in response to these stimuli, and they encoded stimulus intensity better. Comparison of the normalized response profiles of all 28 nociceptive lamina I STT neurons, independent of cell classification, revealed 2 subgroups that differed significantly: “Maintained” cells with responses that remained above 50% of the initial peak rate during stimulation and “Adapting” cells with responses that quickly declined to <50%. The Maintained neurons encoded the intensity of the mechanical stimulus better than the Adapting neurons, based on ratiometric functions. A k-means cluster analysis of all 28 cells distinguished the identical two subgroups. These categories corresponded closely to the NS and HPC categories: Maintained cells were mostly NS neurons (10 NS, 3 HPC), and Adapting cells were mostly HPC neurons (4 NS, 11 HPC). Thus the present data are consistent with the distinctions between A-fiber and C-fiber nociceptors observed previously, because A-fiber nociceptors are the predominant input to NS lamina I STT neurons and C-fiber nociceptors are the predominant input to HPC neurons. These findings support the view that NS, but perhaps not HPC, lamina I STT neurons have a role in the pain caused by maintained mechanical stimuli and contribute to the sensations of “first” pain and “sharpness.” Nonetheless, none of the units studied showed increasing responses during the stimuli, suggesting a role for other ascending neurons or forebrain integration in the augmenting pain produced by maintained mechanical stimulation.

INTRODUCTION

Pain can be caused by mechanical, thermal, or chemical stimulation of the skin and other tissues. The sensations evoked by these submodalities clearly differ, yet the physiological mechanisms underlying these sensations have not been differentiated. Lesions of the lateral spinothalamic tract (STT) produce profound loss of sensation to each of these submodalities (see Craig 2000), and studies of spinothalamic tract neurons and dorsal horn cells have shown that many nociceptive neurons respond to most or all of these submodalities (see Carstens 1997; Willis and Westlund 1997), yet only within lamina I of the spinal dorsal horn have different types of modality-selective STT neurons been identified (Andrew and Craig 2001; Craig and Kniffki 1985; Craig et al. 2001). The available evidence supports the concept that the lamina I STT projection comprises several discrete ascending sensory channels that could provide the basis for distinct sensations (Craig et al. 2001). In this and the following paper (Craig and Andrew 2002), we report experiments designed to distinguish the possible contributions of subsets of nociceptive lamina I STT neurons to different aspects of cutaneous pain sensation.

A sustained, noxious mechanical stimulus produces a perception of pain in humans that continues throughout the stimulus and grades with the intensity of the stimulus (Adriansen et al. 1984; Andrew and Greenspan 1999). In a recent study using a systematically designed set of mechanical probes, psychophysical pain judgments in humans were compared directly with primary afferent nociceptor discharges recorded in rats during maintained mechanical stimulation, and the characteristics of mechanically responsive A- and C-fiber nociceptors were found to differ (Andrew and Greenspan 1999). A-fiber nociceptors displayed responses that were maintained throughout the stimulus and that reliably distinguished the forces and probe sizes, whereas C-fiber nociceptors had responses that adapted quickly and did not distinguish force or probe size as well.

In the present experiments, we used a subset of the stimuli used by Andrew and Greenspan (1999) to examine lamina I STT cells, and thus the present data can be directly compared with the earlier psychophysical and nociceptor responses, as well as with other psychophysical (Greenspan and McGillis 1991, 1994) and single fiber recording (Garell et al. 1996; Slugg et al. 2000) results from similar studies of mechanical pain. The present data indicate that two distinguishable subgroups of nociceptive lamina I STT neurons display either maintained or adapting discharges, respectively, during nox-
ious mechanical stimuli and reflect the distinct response patterns of A- and C-fiber nociceptors observed previously. These data suggest that nociceptive-specific (NS), but not polymodal nociceptive (HPC, for heat, pinch, and cold), lamina I STT cells have a particular role in the sensations of “sharpness” and “first” pain. In contrast, the data presented in the following article indicate that HPC cells, but not NS cells, can be associated with a particular role in “second” or “burning” pain (Craig and Andrew 2002). A preliminary report has been given (Andrew and Craig 1999).

METHODS

Anesthesia and preparation of animals

Experiments were performed on 19 adult cats (2.4–4.1 kg) that were anesthetized with pentobarbital sodium (Nembutal 40 mg/kg ip; Abbott, N. Chicago, IL). Anesthesia was maintained with additional doses (5–10 mg·kg⁻¹·h⁻¹) of pentobarbital given via a cannula in the left cephalic vein. To prevent edema, 10 mg of dexamethasone was given intravenously. Cannulae were also placed in the left carotid artery and in the trachea. Systemic blood pressure was recorded with a pressure transducer connected to the arterial cannula. Core temperature was maintained at 37.5°C with a heating blanket and an infra-red lamp that was thermostatically controlled from a rectal thermistor. The animals were paralyzed with Pancuronium bromide (400 µg iv; Elkins-Sinn, Cherry Hill, NJ) and ventilated with a mixture of 60% O₂ and 40% air using a positive-pressure respirator. Tidal volume and respiratory rate were adjusted to maintain end-tidal CO₂ between 3.8% and 40% air using a positive-pressure respirator. Tidal volume and respiratory rate were adjusted to maintain end-tidal CO₂ between 3.8 and 4.2%. Three indicators of adequate anesthetic depth were monitored during paralysis: 1) the pupils were constricted; 2) blood pressure was stable during noxious stimulation; and 3) when the paralytic agent wore off, as evidenced by muscle twitches during electrical stimulation of the thalamus (see following text), pinching a forepaw did not evoke a withdrawal reflex.

The animal’s head was mounted in a stereotaxic device after topically anesthetizing the ear canals with benzocaine spray (Ceta-caine; Cetylite Industries, Pennsauken, NJ). Additional precautions to limit the nociceptive barrage during the surgical preparation included preventing the corneas from drying with eye salve, and injecting the long-acting local anesthetic bupivacaine prophylactically into the sites of all incisions. A craniotomy was performed to allow vertical microelectrode penetrations into the right somatosensory thalamus, prior to the placement of antidromic stimulating electrodes. The lumbosacral enlargement of the spinal cord was exposed by laminectomy and stabilized with clamps. A pool formed from the surrounding tissues was filled with Tyrode’s solution, and its temperature was maintained at 38°C with a heating coil.

At the end of each experiment, the animal was killed with an overdose of anesthetic. All of the experimental protocols were approved by the local Institutional Animal Care and Use Committee, and they conform to the guidelines of the American Physiological Society and the National Institutes of Health.

Placement of antidromic stimulating electrodes

In each animal an array of six bipolar stimulating electrodes (NE-100; Rhodes Medical Instruments, Woodland Hills, CA) was inserted into the right thalamus, to stimulate the terminals of lamina I STT neurons (Craig 1991; Craig and Dostrovsky 1991). To determine the correct coordinates for the array, a detailed electrophysiological mapping of the ventrobasal thalamus was performed. Multi-unit recordings of somatosensory activity were made with a glass-insulated tungsten microelectrode (30- to 40-µm exposed tip) in response to tapping or stroking the contralateral hemi-body. The first electrode track was made at the stereotaxic coordinates AP +9.5, ML 6.0, and subsequent penetrations were made further posteriorly and medially to map the representations of the forepaw, face, and mouth. Once neurons with ipsilateral intraoral receptive fields were found, the electrode was moved in 0.25-mm steps to locate a group of cells in the dorsomedial aspect of the ventral posterior medial nucleus (dmVPM) that responded to cooling of the ipsilateral tongue (Langdren 1960). The site of these cooling-sensitive cells allowed the location of other lamina I termination sites to be extrapolated, based on prior anterograde tracing and antidromic mapping studies (Craig 1991; Craig and Dostrovsky 2001). The positions of the tips of the electrodes in the array were confirmed histologically in some cases. Of the six electrodes in the array, two were aimed at nucleus submedius (Sm), one targeted the ventral periphery of the basal part of the ventral medial nucleus (VMB), one targeted the cooling-responsive region in dmVPM, one was aimed at the ventral posterior inferior nucleus (VPI), and the final electrode targeted the ventral periphery of the ventral posterior lateral nucleus (VPL) (see Craig et al. 2001).

Identification and classification of lamina I STT cells

Platinum-plated, glass-insulated tungsten microelectrodes (15- to 20-µm exposed tip) were used to record from neurons in the superficial dorsal horn of the L₇ and S₁ segments of the spinal cord. Electrode penetrations were made close to the dorsal root entry zone. Lamina I was found just below a region of group I fiber activity and was generally identifiable as a thin (~200 µm) zone where multi-unit discharge in response to cooling the skin with wet ice could be evoked. Spinothalamic neurons were identified by their antidromic responses to electrical stimulation of the contralateral thalamus with the implanted electrode array. The search stimulus was a train of three bipolar pulses (2 mA intensity, 2 ms duration, 150–200 Hz, center pole negative) delivered in turn from each of the six stimulating electrodes. The position of the recording electrode was adjusted to isolate a single neuron, on the basis of spike amplitude. Each unit isolated was identified as an STT neuron if it fulfilled the following criteria: 1) an all-or-none response at constant latency in response to suprathreshold antidromic stimulation; 2) the ability to follow a train of six antidromic stimuli at 250 Hz (Fig. 1A); and 3) collision between an orthodromic and an antidromic impulse within the critical interval (Fig. 1B). The conduction distance from the stimulating electrodes in the thalamus to the recording electrode in the spinal cord was measured at the end of each experiment.

Units were classified as one of three functional types (Craig and Knifki 1985; Craig and Serrano 1994; Craig et al. 2001) based on their responses to the following forms of cutaneous stimulation: innocuous cooling, innocuous warming, innocuous and noxious mechanical stimuli, noxious heat, and noxious cold stimuli. Cells that responded maximally to innocuous cooling and whose ongoing activity was inhibited by warming were classified as cooling-sensitive thermoreceptive (COOL) cells. Units that showed phasic responses to innocuous cooling and tonic responses to noxious cold, in addition to being responsive to noxious heat and noxious mechanical stimulation were classified as polymodal nociceptive (HPC, for heat, pinch, and cold) cells. Neurons that were responsive to noxious mechanical and/or noxious heat stimuli, but were unresponsive to cold stimuli were classified as nociceptive-specific (NS). There are a few wide dynamic range neurons in lamina I of the cat’s spinal cord that are sensitive to both innocuous and noxious mechanical stimuli, but their axons do not usually project as far as the thalamus (Craig and Serrano 1994), and none were recorded in the current experiments. Prior to detailed study, the background discharge rate of each cell was recorded for 2 min in the absence of stimulation; the receptive field of each unit was mapped manually after this period using suprathreshold mechanical and/or thermal stimuli.

The NS and HPC lamina I STT cells were further characterized with quantitative thermal stimuli (see Craig et al. 2001). To ensure that recordings from the same unit were maintained throughout the
characterization of a unit’s receptive properties, its waveform was constantly monitored and its identity repeatedly checked by thalamic stimulation to confirm that its antidromic latency remained the same. Standard thermal stimuli were applied with a computer-controlled thermoelectric Peltier element (area 16 cm²) placed on the receptive field of the unit. Recordings of temperature were obtained from a thermocouple fixed to the Peltier element. Cooling stimuli were of 20 s duration and were delivered in a descending staircase protocol in steps of 4°C from an adapting temperature of 34°C to a final skin-thermode interface temperature of 12.5°C. Heat stimuli were also of 20 s duration but were delivered as discrete ramp-and-hold (ramp rate 15°C/s) steps from an adapting temperature of 34°C to a final skin-thermode interface temperature of 42°C in 4°C steps. The interval between successive heat stimuli was 60 s.

Quantitative mechanical stimulation

For the present study, mechanical stimuli were applied with pre-weighted probes of constant tip area (0.1 mm²). The probes were attached to a stainless steel rod (1.5 mm diam, 140 mm long) that extended out from the barrels of three 20-ml syringes. Inside the syringes the rod was loaded with metal washers to a final weight of 25, 50, or 100 g. A male Luer fitting was fixed to its free (lower) end. Removable probe tips were made by cementing short lengths of stainless steel rod (0.36 mm diam) with smooth flat ends into 22-gauge hypodermic tubing, each attached to a female Luer fitting. The completed probes applied stimuli of constant intensity (25, 50, or 100 g) over a constant area (0.1 mm²) and were able to accommodate tissue displacements of up to 15 mm. The probes were held in a micromanipulator and applied manually to a unit’s receptive field; a footswitch was used to indicate the time of contact of the probe with the skin. The probes were always applied to the most mechanically responsive part of the receptive field. Three or four short-duration (1–2 s) standardized pinch stimuli (1,000 g) applied with a pair of smooth-tipped forceps (area 3 mm²) were used to locate the part of a unit’s receptive field with the greatest mechanosensitivity; a strain gauge mounted on one of the blades of the forceps was used to obtain a record of the stimulus (modeled after stimulator 2 in Fig. 2 of Burgess and Perl 1967; see Craig et al. 2001). Mechanical stimuli were always applied at the same spot, and trials were separated by 10–20 min to mitigate sensitization and/or fatigue. Generally only 1 or 2 units were studied per experiment using these stimuli, with subsequently characterized neurons having separate receptive fields.

The recording sites of neurons were marked with an electrolytic lesion (+20 μA, 10 s). Segments of spinal cord containing the lesions and thalamic blocks containing the tracks of the stimulating electrodes were fixed in 10% buffered Formalin, and the recording and stimulating sites were identified in 50-μm transverse, thionin-stained sections.

Data capture and statistical analysis

Conventional oscilloscopic and audio displays of the electrophysiological data were used. Data were also digitized with a computer interface (Power1401; Cambridge Electronic Design, Cambridge, UK) for later off-line analysis. Neural records were sampled at 25 kHz, and stimulus records were sampled at 1 kHz.

Statistical analyses were performed with the program Statistica (Statsoft; Tulsa, OK), using both parametric and nonparametric tests as appropriate. To compare the discharge profiles of HPC cells and NS cells to one another, mean firing rates of individual units were computed using 10-s bins. Comparisons were made using absolute firing rates or data normalized to the firing rate during the first 10-s bin after stimulus onset. Trend analysis (a repeated-measures ANOVA that included a specific test for a linear trend over time) was used to evaluate the effects of time on the absolute discharge rates of neurons. The Kruskal-Wallis ANOVA followed by the Kolmogorov-Smirnov two-sample test post hoc were used to test for differences in the normalized discharge profiles of different groups of units to a particular stimulus. A k-means cluster analysis was used to identify statistically different subclasses of neurons. Two-factor ANOVA with a repeated measure on one factor (stimulus intensity) was used to compare stimulus-response functions of different classes of neurons. Simple linear regression analyses were performed using the $y = ax + b$ model, and Spearman’s test was used to investigate correlations. For all statistical tests, $P < 0.05$ was considered significant.
RESULTS

General properties of nociceptive lamina I STT cells

Data were obtained for 28 histologically confirmed (Fig. 1) and antidromically identified lamina I STT cells (14 NS, 14 HPC) in 19 cats. All of the units had receptive fields on the ventral surface of the distal hindlimb. Nineteen units were maximally responsive to stimulation of the glabrous skin, whereas the remaining 9 had receptive fields in hairy skin. Of the 14 NS units, 5 were excited only by noxious mechanical stimulation, whereas 9 responded to both noxious heat and noxious mechanical stimuli. All of the HPC neurons responded to noxious heat, pinch, and noxious cold stimuli, albeit to varying degrees. We have previously shown that NS and HPC neurons differ significantly in their central conduction velocities and their level of background activity (Andrew and Craig 2001; Craig et al. 2001), and the present data were consistent with this. Thus the mean central conduction velocity of the axons of NS cells, calculated by dividing the measured conduction distance by the latency of the electrically evoked antidromic action potential of each unit, was significantly slower (3.4 ± 1.0 m/s, mean ± SD; range 2.4–5.1) than that of the HPC neurons (5.9 ± 1.2 m/s; range 4.7–8.3; P < 0.0002, unpaired t-test). Also, the mean ongoing (background) firing rate of the entire NS population over a 2-min period of recording was lower (0.4 ± 0.6 impulses/s; range 0–1.8) than the mean background firing rate of all 14 HPC neurons (0.8 ± 0.9 impulses/s; range 0–2.3) but not significantly so (P < 0.2; unpaired t-test). The receptive field sizes of NS and HPC neurons were also consistent with prior reports (e.g., Craig and Kniffki 1985; Craig and Serrano 1994) and are not addressed in this report. All of the thalamic nuclei targeted by the array were effective antidromic stimulating sites for the lamina I

FIG. 2. Representative responses from a nociceptive-specific (NS) lamina I STT neuron to mechanical stimulation with a probe of 0.1 mm² tip area for 2 min at intensities of 25 g (A), 50 g (B), and 100 g (C). The discharge of the unit was histogrammed using 1-s bins. D: the superimposed responses at each stimulus intensity. E: the stimulus-response functions of the unit to graded cold (top pair of traces) and graded heat (bottom pair of traces). For each pair of traces, the top record is the firing rate of the neuron displayed in 1-s bins, and the bottom record is the temperature at the skin-thermode interface. The unit had a central conduction velocity of 2.8 m/s, and it projected only to Sm.
STT neurons reported here, although it was evident, based on thresholds and the incidence of antidromically activated units, that in some experiments the array was not well positioned. Twenty-two neurons were activated from the ventral periphery of the ventrobasal thalamus, and 12 of them were also activated from the submedial nucleus. Six neurons were only activated from the submedial nucleus. There were no significant differences between the patterns of effective stimulating sites for NS and HPC neurons \((P > 0.4, \chi^2\text{ test})\) (see Craig and Dostrovsky 2001).

**Characteristics of the responses of nociceptive lamina I STT cells to mechanical stimulation**

Figures 2 and 3 show the responses of an NS lamina I STT neuron and an HPC lamina I STT neuron, respectively, to graded, maintained noxious mechanical stimulation. The responses of these neurons to the standard thermal stimuli that were used to verify their classification are also shown. These individual examples document the differential representation of adaptation rate and intensity-dependent gradation of discharge that distinguished NS and HPC neurons.

The mean responses of the 14 NS units and the 14 HPC units at all 3 stimulus intensities (Fig. 4) similarly reflect these features. The mean discharge of the NS neurons was maintained over the 2-min duration of the mechanical stimuli, whereas the HPC neurons responded to the same stimuli with an initial dynamic component that quickly adapted to a lower level of static discharge. In addition, the responses of the NS neurons to the three intensities of stimulation were clearly graded, whereas those of the HPC neurons were not. Figure 4 also shows that the NS neurons had a modestly greater absolute discharge than HPC neurons; the mean total response to the 100-g stimulus for the NS neurons was 514 ± 356 impulses (range 113–1,154), whereas the corresponding figure for HPC neurons was 280 ± 237 (range 70–957; \(P = 0.05\), unpaired \(t\)-test). Trend analysis showed that the discharge of both the NS and HPC neurons declined over time \((P < 0.0001, \text{ANOVA})\); however, the mean discharge profile of NS neurons adapted...
significantly less than that of the HPC neurons; the mean time constant (decay to 63%) of the NS population response to the 100-g stimulus was 15.9 s, whereas that of the HPC population was 9.5 s ($P < 0.03$, unpaired $t$-test). Significant relationships were also found for the HPC neurons between background activity and peak discharge rate ($r = 0.53$, $P < 0.04$, Spearman’s correlation) and between background activity and total discharge ($r = 0.63$, $P < 0.002$, Spearman’s correlation), but not for the NS neurons (peak rate: $P < 0.8$, Spearman’s correlation; total discharge: $P < 0.4$, Spearman’s correlation), presumably because the background activity rates for NS neurons were generally near zero (Fig. 5, B and C).

The peak discharge rate and the total discharge were correlated for both the NS neurons and the HPC neurons (Fig. 5B). A linear regression for the NS neurons yielded $r = 0.87$ ($P < 0.006$, Spearman’s correlation), and a regression of total and peak HPC discharge yielded $r = 0.75$ ($P < 0.04$). Significant relationships were also found for the HPC neurons between background activity and peak discharge rate ($r = 0.53$, $P < 0.04$, Spearman’s correlation) and between background activity and total discharge ($r = 0.63$, $P < 0.002$, Spearman’s correlation), but not for the NS neurons (peak rate: $P < 0.8$, Spearman’s correlation; total discharge: $P < 0.4$, Spearman’s correlation), presumably because the background activity rates for NS neurons were generally near zero (Fig. 5, B and C).

FIG. 4. Mean responses for all NS (A, $n = 14$) and HPC (B, $n = 14$) lamina I STT neurons to mechanical stimuli delivered at 25, 50, and 100 g intensity. Only every 10th error bar (1 SD) is shown for clarity. The stimuli are indicated by the marker trace.

FIG. 5. A: histogram showing the distribution of peak firing rates for NS and HPC neurons during 2-min stimulation at 100-g intensity with a 0.1-mm$^2$ probe. Bin size, 1 impulse/s. B: relationship between peak discharge evoked by the 100-g, 2-min stimulus and the total discharge for nociceptive-specific (NS) and polymodal nociceptive (HPC) lamina I STT neurons. C: distribution of peak firing rates plotted as a function of ongoing (background) activity for nociceptive-specific (NS) and polymodal nociceptive (HPC) lamina I spinothalamic neurons.
Differentiation of subgroups of lamina I STT neurons with distinct response profiles to mechanical stimuli

Because the peak firing rates of the NS and HPC neurons overlapped broadly, while the rates of decline in their responses to the maintained mechanical stimuli were significantly different, we compared the responses of all units to the 100-g mechanical stimulus after summing individual firing rates in 10-s bins and normalizing each unit’s response to its rate during the initial 10-s bin. This enabled the response profiles of the units to be compared independently of absolute firing rates and unit classification. The initial 10-s bin immediately following stimulus onset was used for normalization, because most (23/28) neurons achieved their maximum firing rate at this time. The data for all 28 lamina I STT neurons are shown in Fig. 6. Two subgroups could be clearly distinguished based on their firing rates 80 s after stimulus onset (at the gap indicated by the asterisk in Fig. 6). At this latency, the median normalized discharge rate of the neurons firing at >50% was almost twice as great as the median rate of the neurons firing at <50% (60.0 vs. 30.4%; P < 0.002, Mann-Whitney U test). Measured at the same time point, the NS neurons had significantly greater normalized discharge rates (median 44.0%, 25th percentile 22.8, 75th percentile 62.8) than the HPC neurons (median 27.3%, 25th percentile 14.6, 75th percentile 33.3; P < 0.04, Mann-Whitney U test), although the difference between their absolute discharge rates at 80 s did not achieve significance (P < 0.2, unpaired t-test).

Exactly the same two groups were independently identified, one-for-one, with a k-means cluster analysis (P < 0.003) of the population of normalized responses. (Additional partitions could be identified with cluster analyses that split these 2 groups based on different rates of response decline. Cluster analyses based on absolute discharge rates instead of normalized rates also identified up to 4 clusters of neurons, but these clusters were distinguished primarily by their absolute firing rates at stimulus onset and not by their discharge profiles.)

One group, identified by both the separation of the normalized response profiles at the 80 s time bin and by the cluster analysis was classified as “Maintained” (discharge >50% at 80 s) and the other was classified as “Adapting” (discharge <50% at 80 s). The 13 cells classified as Maintained included 10 NS and 3 HPC lamina I STT cells (Fig. 7A), and their normalized firing rates remained above 50% of maximal throughout the duration of the stimulus (median 60.4% during time bins 60–120 s after stimulus onset). In contrast, the 15 Adapting units, which included 4 NS and 11 HPC lamina I STT cells (Fig. 7B), had responses in which the initial dynamic response following stimulus onset was followed by firing at a lower rate (median 26.3% during time bins 60–120 s after stimulus onset). Because these were normalized data, the non-parametric Kruskal-Wallis ANOVA was used to determine whether the temporal profiles of the Maintained neurons were significantly different from those of the Adapting neurons, and the Kolmogorov-Smirnov two-sample test was used post hoc to test for differences at individual 10-s intervals during stimulation. These two groups had significantly different discharge profiles (P < 0.0001, Kruskal-Wallis ANOVA), with the Maintained units having significantly greater normalized firing rates than the Adapting units at times 30–130 s after stimulus onset (Fig. 7, C and D; P < 0.005 at each time point). Trend analysis showed that the normalized discharge of the Adapting neurons declined significantly during mechanical stimulation (P < 10^-3, ANOVA), whereas there was no significant decline in the discharge of the Maintained neurons (P > 0.7, ANOVA).

The relationship between stimulus intensity and firing rate was investigated for Maintained and Adapting neurons using the ratiometric method described by Cervero et al. (1988). With this method, the response (total impulse count) evoked by the weakest stimulus (25 g in the current experiments) was normalized to 1, and subsequent responses to the more intense stimuli were expressed as a multiple of this ratio (Cervero et al. 1988). Maintained units encoded stimulus intensity significantly better than the Adapting units (Fig. 8; P < 0.005 Kruskal-Wallis ANOVA, P < 0.005 Kolmogorov-Smirnov test post hoc).

Given that most of the units with Maintained response profiles to noxious mechanical stimuli were NS neurons (10/14) and most of the units with Adapting profiles were HPC neurons (11/14), there were corresponding differences between these cell groups. Maintained neurons were generally insensitive to cooling or cold stimuli (e.g., Fig. 2E), whereas Adapting neurons usually responded to noxious cold stimuli (e.g., Fig. 3E). Maintained neurons also had lower rates of background (ongoing) activity than Adapting neurons (Maintained: mean = 0.2 ± 0.5 imp/s; Adapting: mean = 0.9 ± 0.8 imp/s; P < 0.004, unpaired t-test) and the heat-evoked stimulus-response functions of Maintained neurons were not significantly different from those of Adapting neurons (P > 0.3; 2-factor repeated measures ANOVA, Fig. 9B); and, the thalamic sites from which the neurons were antidromically activated did not differ (P > 0.1, χ² test).

![FIG. 6. Normalized discharge profiles evoked using the 100-g stimulus for all lamina I STT neurons studied. Mean firing rates were computed for 10-s bins and normalized to the mean firing rate during the initial 10-s bin immediately following stimulus onset. At the asterisk (*), note the separation of the curves at time 80 s. NS neurons had significantly greater normalized firing rates than HPC neurons during this 80-s bin (P < 0.04, Mann-Whitney U test).](Image)
DISCUSSION

Three important findings emerge from the present experiments. First, the responses of NS lamina I STT and HPC lamina I STT neurons to maintained noxious mechanical stimuli differ, with the absolute discharges of NS neurons showing less adaptation and more maintained profiles that better resemble the psychophysical data (Adriansen et al. 1984; Andrew and Greenspan 1999) than the responses of HPC cells, and with only NS neurons showing graded responses that encode all stimulus intensities. Second, independent analysis of normalized response profiles revealed a Maintained group of lamina I STT neurons with discharge profiles that do not decline during the 2-min mechanical stimulus and that encode intensity ratio metrically, in contrast to the group of neurons with Adapting profiles. Last, the categories NS and HPC correspond largely to the categories Maintained and Adapting. The properties established for these neurons are compared in the following text to prior data obtained from primary afferent and central neuron recordings and from psychophysical experiments in humans.

Comparison with prior primary afferent recordings

Little is known about the characteristics of primary afferent activity or central neural encoding of noxious mechanical stimuli. Mechanical thresholds, as determined with von Frey hairs, are usually reported, but this measure provides little in the way of quantitative data, because there is an uneven tradeoff between force and surface area as the hairs become stiffer, and because as the hair is bent the contact area of stiffer hairs changes and generates edge effects. Thus the use of a systematic set of force-controlled stimuli is required, such as previously used by others to compare nociceptor activity with human sensation (Andrew and Greenspan 1999; Garell et al. 1996; Greenspan and McGillis 1991, 1994; Slugg et al. 2000). Those studies indicate that A-fiber nociceptors encode the...
spatial and intensive aspects of noxious mechanical stimuli considerably better than C-fiber nociceptors, and that A-fiber nociceptors generally have stimulus-response curves that relate better to human pain thresholds than C-fibers. Two populations of A-fiber nociceptors with different mechanical response properties have been distinguished (Andrew and Greenspan 1999), one with monotonic, graded stimulus-response functions to graded mechanical stimuli and more maintained (slowly adapting) responses to maintained stimuli, and another that adapted quickly and showed a plateau at stimulus intensities close to human pain threshold. The responses of the former group was better related to human pain judgments than that of the latter group. The discharge of both groups of A-fiber nociceptors declined over time during mechanical stimulation, but the former group showed less adaptation (to approximately 30% of the initial maximal discharge rate after 80 s) than either the latter group of A-fiber nociceptors or the C-fiber nociceptors (to approximately 10% of the initial maximal rate after 80 s), albeit not sufficient to explain the augmenting human pain sensation with maintained mechanical noxious stimulation (Adriansen et al. 1984; Andrew and Greenspan 1999).

The present data show that NS lamina I STT neurons seem to reflect particular input from the slowly adapting type of A-fiber nociceptor, but in contrast to those fibers, the NS neurons had responses that were clearly maintained during the entire stimulus, as well as showing graded responses that encoded stimulus intensity. Furthermore, the Maintained subgroup, which comprised most of the NS cells, showed discharges that remained above 50% throughout the stimulus, and this clearly exceeds the discharge profiles of peripheral nociceptors. On the other hand, the responses of the Adapting (or, HPC) lamina I STT neurons to mechanical stimulation were similar to those of C-fiber polymodal nociceptors, which are the predominant input to this class of neurons (Craig and Kniffki 1985; Craig et al. 2001), and they were also similar to the responses of the rapidly adapting class of A-fiber nociceptors identified in rats (Andrew and Greenspan 1999), indicating that they receive little, if any, input from the more slowly adapting A-fiber nociceptors.

The present observations demonstrate that the activity of nociceptive lamina I STT neurons closely resembles but does not simply mirror the responses of the primary afferent fibers, indicating that there is selective afferent convergence and some temporal integration, albeit not sufficient to solely account for the prior psychophysical data. These observations are consistent with prior evidence of selective primary afferent convergence and temporal integration in lamina I STT neurons (Craig and Andrew 2002; Craig et al. 2001).

Comparison with prior lamina I neuron recordings

The present study is the first to use force-controlled stimuli to study central nociceptive neurons. In recent studies, manually applied, force-monitored mechanical stimuli with large probes were used to examine lamina I spinoparabrachial neurons in rat (Bester et al. 2000) and lamina I spinothalamic neurons in cat (Craig et al. 2001). In earlier studies, graded stimulation using von Frey hairs was used to characterize one lamina I trigeminothalamic neuron (Price et al. 1976) and several primate spinothalamic neurons of unspecified location (Palecek et al. 1992).

In the only prior quantitative study that differentiated NS and HPC lamina I STT cells, NS cells were found to have a noticeably lower mechanical threshold than HPC cells, but otherwise their mechanical responses to staircase force stimuli were quite similar (Craig et al. 2001). Most previous investigations failed to distinguish NS and HPC lamina I neurons, because cold stimuli were seldom used in characterizing unit receptive properties (see Han et al. 1998). Cervero et al. (1976, 1979) classified nociceptive lamina I neurons as class 3a neurons that selectively responded to noxious mechanical stimulation and only received inputs from myelinated (Aδ) nociceptors, or class 3b neurons that responded to noxious mechanical and noxious heat stimuli and received inputs from both myelinated and unmyelinated (C-fiber) nociceptors. Our previous studies have shown that NS lamina I STT neurons receive strong A-fiber inputs and weak or absent C-fiber inputs, whereas HPC neurons receive strong, monosynaptic C-fiber inputs and weaker A-fiber inputs (Craig and Kniffki 1985; Craig et al. 2001). At first glance the 3a/3b classification scheme appears comparable with our NS/HPC classification scheme, but the HPC neurons are distinguished as well by their cold sensitivity (e.g., Fig. 3E), which Cervero and colleagues did not test. Additionally, the central conduction velocities of NS lamina I STT neurons are slower than those of HPC.
neurons, whereas the reverse is true for Class 3a/3b lamina I neurons. However, Cervero and colleagues measured the conduction velocities of class 3 neurons with electrodes placed ipsilaterally in Lissauer’s tract, rather than in the contralateral thalamus, as was done in the present experiments.

Most of the Maintained neurons were NS neurons, and most of the Adapting neurons were HPC neurons. Whereas the mean response characteristics of NS neurons closely resembled those of the Maintained group, and the characteristics of HPC neurons closely resembled those of the Adapting group, a small number of NS neurons (4/14) and a small number of HPC neurons (3/14) had opposite characteristics, i.e., NS neurons with adapting profiles and HPC neurons with maintained profiles. Although the NS and HPC categories are robust and these two classes of neurons can be reliably differentiated based on other criteria, such as central conduction velocity, ongoing activity, qualitative and quantitative thermal response characteristics, and cell shape (Andrew and Craig 2001; Craig et al. 2001; Han et al. 1998), the present findings suggest that there may be subclasses within each of these groups with different mechanical encoding properties, just as there are subclasses of A-fiber and C-fiber nociceptors with different stimulus-response functions (Andrew and Greenspan 1999; Garell et al. 1996). Similarly, Craig et al. (2001) recently identified subclasses of COOL and HPC neurons with different cooling-evoked stimulus-response functions that probably reflect selective input from different subclasses of primary afferents. Furthermore, prior analyses of lamina I STT cells clearly indicated that subtypes of the three major morphological categories (fusiform NS, pyramidal COOL, and multipolar HPC neurons) exist, as well as cells with transitional shapes (Han et al. 1998; Zhang and Craig 1997) or transitional physiology (Craig et al. 2001), as might be expected in a developmentally defined neurobiological system. Further experiments using force-controlled mechanical stimuli (Andrew and Greenspan 1999; Garell et al. 1996; Greenspan and McGillis 1991, 1994) are needed to characterize such units more completely.

A quantitative relationship was found between a unit’s peak discharge rate and its total response to a maintained mechanical stimulus. This has not been reported for lamina I STT cells, but could be predicted on the basis of primary afferent recordings (Andrew and Greenspan 1999; Handwerker et al. 1987) and the response patterns of lamina I cells. A correlation of greater possible significance was found between ongoing background activity and the peak discharge rate and total discharge for HPC neurons; this was not found for NS neurons, perhaps because they have significantly lower rates of background activity (Andrew and Craig 2001; Craig et al. 2001). The linear relationships between ongoing activity and both peak and total discharge likely reflect a common source in primary afferent activity, consistent with the possibility that the ongoing activity of HPC neurons is due to ongoing discharge in the primary afferent C-fibers that converge on such cells. Ongoing discharge has been reported in many C-fibers from muscle (Mense and Meyer 1985) and joint (Schaible and Schmidt 1983), although little has been reported from skin. Yet, low rates of ongoing discharge in C-fibers would not necessarily (under normal circumstances) generate a sensation, for which central summation of C-fiber activity is required (Adriansen et al. 1984; Gybels et al. 1979), and they may not be easily observable. Even a low level of background activity in cutaneous C-fiber afferents, such as only 1 or 2 impulses per minute per fiber, could generate the level of background activity observed in HPC cells, because hundreds of C-fibers must converge on each single HPC neuron. Significantly, distinct levels of ongoing discharge are found in each distinct class of lamina I STT cells (Craig et al. 2001); for example, in stark contrast to HPC cells, histamine-selective lamina I STT cells have zero ongoing discharge, like their afferent C-fibers (Andrew and Craig 2001). We suggest that if the ongoing discharge of HPC lamina I neurons is due to ongoing activity in their primary afferent C-fiber input, then this probably has biological significance, consistent with the concept that the lamina I projection system serves as an interoceptive (homeostatic) afferent pathway (Craig 1996, 2000; see Craig and Andrew 2002). The present findings indicate that this possibility needs to be tested.

**Lamina I STT neurons and mechanical pain**

The present findings allow us to consider the contribution of different classes of nociceptive lamina I STT neurons to mechanically evoked pain. A single noxious mechanical stimulus was shown to evoke two pain sensations by Lewis and Pochin (1937). They obtained evidence to suggest that the two pains were conducted by different classes of afferents; one group with rapidly conducting fibers (“first” pain) and the other with much more slowly conducting axons (“second” pain). Earlier studies had established that myelinated A-fibers conduct pain-related activity that produces sensations with a sharp pricking or stinging painful quality (Collins et al. 1960; Heinbecker et al. 1933). Subsequent nerve block and latency measurement studies concluded that A-fiber nociceptors are associated with sharp, pricking (first) pain, whereas C-fiber nociceptors are associated with affectively strong, burning (second) pain sensations (Campbell and LaMotte 1983; Muckenzie et al. 1975; Torebjörk and Hallin 1973). Microstimulation of presumed single nociceptors in human microneurography studies confirmed these earlier studies (Konietzny et al. 1981; Ochoa and Torebjörk 1989; Schady et al. 1983).

Noxious punctate stimuli, such as those used here, predominantly evoke sharp pricking painful sensations typical of first pain (Andrew and Greenspan 1999). In contrast to the periphery, where the relationship between afferent activity and pain sensation has been described, comparatively little is known about the central mechanisms of first pain. The Maintained (or, NS) class of lamina I STT neurons could provide a central substrate for first pain, because they have better mechanical encoding properties than the Adapting (or, HPC units) neurons, because their discharge profiles to mechanical stimuli more closely match the psychophysical data than the profiles of HPC neurons do, and because 5 of the 14 neurons studied were modality-specific in that they only responded to noxious mechanical stimulation. A noxious heat stimulus can also evoke a first pain sensation, described as “sharp” or “pricking heat” earlier and at lower temperatures than the sensation of “burning” (Boring 1942; Campbell and Meyer 1996), and this early pricking heat sensation is also conducted by myelinated nociceptors (Campbell and LaMotte 1983; Treede et al. 1995). This is consistent with the observation by Craig et al. (2001) that the thresholds of NS lamina I STT neurons for noxious heat have a significantly lower distribution (median ~43°C) than the HPC neurons (median ~45.5°C). Thus despite the fact that
many NS neurons are heat responsive, their main role may be in relation to first pain. In contrast, our data indicate that the HPC lamina I STT neurons do not have a particular role in first pain but, as described in the following article (Craig and Andrew 2002), they show summating responses to repeated brief contact heat stimuli that parallel the psychophysics of the sensation of second pain (Vierck et al. 1997), whereas NS neurons do not. Nonetheless, HPC lamina I STT neurons are activated by the noxious mechanical stimuli used in this study, albeit briefly and less strongly, and their contribution to mechanical pain depends on how this activity is integrated in the forebrain with the activity of NS lamina I and other nociceptive STT cells.

Additionally, NS lamina I STT neurons may also have a role in the perception of sharpness. Sharpness is evoked by mechanical stimuli that are strong enough to activate A-fiber nociceptors but not intense enough to produce a sensation of pain (Greenspan and McGillis 1991, 1994), and because this perception does not spatially summate, sharpness has been suggested to be due to activity in perhaps individual A-fiber nociceptors (Greenspan et al. 1997). The present data indicate a close similarity in the response patterns of Maintained (NS) lamina I STT neurons and the slowly adapting category of A-fiber nociceptors that are suggested to underlie the sensation of sharpness.

A paradox inherent in the association of NS lamina I STT neurons with first pain is that their central conduction velocities are slower than those of HPC neurons, which we associate with second pain (Craig and Andrew 2002). However, the difference in the central latencies of these two classes of neurons would probably be imperceptible. Most of the delay between first and second pain (700–1,500 ms) (Campbell and LaMotte 1983; Lewis and Pochin 1937) can be attributed to the difference between the conduction velocities of A- and C-fiber nociceptors. Following a suprathreshold heat stimulus, the first action potentials conducted by primate type II A-fiber mechano-heat nociceptors would arrive in the spinal cord within 100–300 ms, whereas the first impulses carried by C-fiber mechano-heat nociceptors would arrive at delays between 700 and 1,400 ms (Treede et al. 1995). Assuming a conduction distance of 50 cm (from the L2 segment of the human spinal cord to the thalamus), and a central conduction velocity of 3.4 m/s for the NS neurons and 5.9 m/s for the HPC neurons (as reported here, although the conduction velocities of lamina I STT neurons in primates are faster) (see Dostrovsky and Craig 1996), then impulses conducted by NS neurons would arrive in thalamus 62 ms later than impulses conducted by HPC neurons. This 62 ms difference is trivial in comparison to the 600–1,100 ms delay due to the difference in A- and C-fiber conduction velocities. Thus, the apparently slower conduction time of first pain in the STT should have little impact on perception.

Tonic mechanical stimulation and augmenting pain sensation

The augmenting pain sensation produced by sustained noxious mechanical stimuli has previously been considered to be due to temporal summation, because the responses of cutaneous A-fiber high-threshold mechanoreceptors and C-fiber mechano-heat (polymodal) nociceptors all decline over time (Adriansen et al. 1984; Andrew and Greenspan 1999; Handwerker et al. 1987). The integration that results in the maintained responses of NS lamina I STT neurons is not sufficient to explain the augmenting human sensation. One possible mechanism of temporal augmentation is a central comparison of the activity of nociceptors and low-threshold mechanoreceptors (Adriansen et al. 1984), resulting in a “pattern” that increases over time, which would be consistent with the “gate control theory” of pain. However, this mechanism seems unlikely, because blocking conduction in either Aβ-fibers, alone or in combination with Aδ-fibers, does not abolish the augmenting pain sensation (Andrew and Greenspan 1999). Symptoms consistent with nociceptive temporal summation such as allodynia and hyperalgesia are exhibited in many human diseases that produce intractable pain, thus the mechanisms of augmenting pain sensations are likely to be of considerable clinical importance (Pagni 1998).

The recent description of mechanically insensitive C-fibers in humans that are activated after an initially silent period and increase their firing rate during maintained pressure stimulation (Schmidt et al. 2000) raises the possibility that the augmenting pain sensations to maintained mechanical stimulation are due to the recruitment of such afferent activity. None of the lamina I STT neurons tested with mechanical stimuli in the current study had discharge profiles that increased over time, suggesting that further central integration of STT activity is needed to account for the augmenting pain sensations. Temporally increasing responses during sustained mechanical stimulation have been described for a subset (23%, 12/52) of rat spinal neurons with unidentified projections (Cervero et al. 1988). However, there was in all likelihood spatial as well as temporal summation in that study since the probe used was of substantially larger tip area (9.6 mm²; 3.5 mm diam) than the one (area 0.1 mm²) used in our study. One possible group of spinothalamic neurons whose activity could be integrated rostrally with the activity of NS and HPC neurons (and also other nociceptive STT neurons in the deep dorsal horn) is the recently described chemo-nociceptive cell class (Andrew and Craig 2001). These neurons receive inputs from mechanically insensitive C-fibers, and although they have not yet been tested with sustained mechanical stimuli, chemicals released at the stimulated site might activate them during such stimuli (cf. Schmidt et al. 2000).

Conclusions

In summary, we have shown that different classes of nociceptive lamina I STT neurons have different responses to maintained noxious mechanical stimuli that reflect similar differences in peripheral nociceptors. The NS neurons encode stimulus intensity better and have responses that decline more slowly than the HPC neurons. These categories correspond essentially to Maintained and Adapting neurons, which are clearly differentiated by their relationship to the pain reports in humans evoked by the same stimuli. This evidence indicates that a particular role in the sensations of first pain and sharpness can be ascribed to NS lamina I STT neurons, which seem to receive a selective input from the slowly adapting subtype of A-fiber nociceptors, with further temporal integration. Nonetheless, subclasses of NS neurons might be distinguishable that...
could ultimately lead to a more complete understanding of lamina I STT neurons. In contrast, the HPC lamina I STT neurons do not seem to contribute to first pain, consistent with their association with second pain as described in the following paper (Craig and Andrew 2002). The present results provide strong evidence differentiating nociceptive NS and HPC lamina I STT neurons and supporting the concept that they comprise several discrete, modality-selective sensory channels that represent distinct aspects of pain as well as temperature and itch.

We thank S. Jordan and M. Tatum for excellent technical assistance. This study was supported by National Institute of Neurological Disorders and Stroke Grant NS-25616 and the Atkinson Pain Research Fund administered by Barrow Neurological Foundation.

REFERENCES


