

Chung-Chih Kuo and Chen-Tung Yen

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Comparison of Anterior Cingulate and Primary Somatosensory Neuronal Responses to Noxious Laser-Heat Stimuli in Conscious, Behaving Rats

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Kuo, Chung-Chih and Chen-Tung Yen. Comparison of anterior cingulate and primary somatosensory neuronal responses to noxious laser-heat stimuli in conscious, behaving rats. *J Neurophysiol* 94: 1825–1836, 2005; 10.1152/jn.00294.2005. In this study, we investigated single-unit responses of the primary sensorimotor cortex (SmI) and anterior cingulate cortex (ACC) to noxious stimulation of the tail of the rat. The influences of morphine on these nociceptive responses were also compared. Multiple single-unit activities were recorded from two eight-channel microwire arrays chronically implanted in the tail region of the SmI and ACC, respectively. CO₂ laser-heat irradiation of the middle part of the tail at an intensity slightly higher than that causing a maximal tail flick response was used as a specific noxious stimulus. Examined individually, ACC neurons were less responsive than SmI neurons to laser-heat stimulus, in that only 51% of the ACC units ($n = 125$) responded compared with 88% of the SmI units ($n = 74$). Among these responsive ACC units, many had a very long latency and long-lasting excitatory type of response that was seldom found in the SmI. When ensemble activities were examined, laser heat evoked both short- (60 ~ 150 ms) and long-latency (151 ~ 600 ms) responses in the SmI and ACC. Latencies of both responses were longer in the ACC. Furthermore, a single dose of 2.5–10 mg/kg morphine intraperitoneally suppressed only the long latency response in the SmI, but significantly attenuated both responses in the ACC. These effects of morphine were completely blocked by prior treatment with the opiate receptor blocker, naloxone. These results provide further evidence suggesting that the SmI and ACC may play different roles in processing noxious information.

INTRODUCTION

Recent neuroimaging studies in human subjects have revealed several key forebrain areas most often activated by noxious stimulations. Among these areas are the primary somatosensory cortex (SI) and anterior cingulate cortex (ACC) (Casey et al. 1994; Coghill et al. 1994, 1999; Craig et al. 1996; Davis et al. 1995; Dorbyshire et al. 1997; Hsieh et al. 1995; Talbot et al. 1991; Vogt et al. 1996). Furthermore, studies by Rainville et al. (1997) and Hofbauer et al. (2001) showed that functional MRI (fMRI) intensities in the SI were correlated with the intensity of the noxious heat, whereas those in the ACC were better correlated with subjective unpleasantness, suggesting a differential functional role between SI and ACC.

In contrast, the processing of nociceptive information in the forebrain at the cellular level is largely unclear. Neuroanatomical and neurophysiological studies have shown that pain information is processed through a complicated serial and parallel network in the forebrain (Craig 2003; Willis 1985). Electrophysiological studies of the SI have revealed nociceptive neu-

rons that have a definite and restricted nociceptive receptive field and are somatotopically organized (Chudler et al. 1990; Kenshalo and Isensee 1983; Kenshalo et al. 2000; Lamour et al. 1983a,b). ACC nociceptive neurons tend to have rather large nociceptive receptive fields and may even include the entire body (Hutchinson et al. 1999; Sikes and Vogt 1992; Yamamura et al. 1996). Thus neurons in the SI and ACC seem to exhibit different response properties.

Most of the electrophysiological studies have been carried out on anesthetized animal preparations. Because pain sensations are abolished by general anesthesia, the relevance of results from subjects in an anesthetized condition to pain function is unclear. In the studies performed in unanesthetized human subjects (Hutchinson et al. 1999) or monkeys (Kenshalo et al. 1988), on the other hand, either ACC or SI units were respectively recorded, and very different sensory stimuli were tested. Thus quantitative comparisons cannot be made. It would be of interest to test the neuronal responses of the SI and ACC in the same subject with the same array of specific noxious stimuli. To address this critical issue, the first goal of this investigation was to simultaneously record SI and ACC neuronal responses to laser-heat stimulation applied to the mid-part of the tail of conscious rats.

Morphine has powerful analgesic effects. The amplitude of the laser-heat-evoked potential (LEP) is reduced by morphine in parallel with marked clinical pain relief (Kalliomaki et al. 1998; Lorenz et al. 1997). Moreover, Tsai et al. (2004) reported that morphine suppresses only the long-latency laser-evoked responses but not the short-latency laser-heat-evoked unit responses in the rat SI. With relatively denser concentrations of endogenous opiates and opiate receptors in the limbic cortex (Jones et al. 1991; Lewis et al. 1983; Mansour et al. 1986, 1987), it would be of interest to compare the effects of morphine on SI and ACC nociceptive responses such that sensory and affective aspects of the pain function may be explored pharmacologically (Merskey 1986). Our preliminary results were previously reported (Kuo and Yen 2003).

METHODS

Experiments were performed on eight female Long-Evans rats weighing 220 ~ 300 g at the time of electrode implantation. The care of the animals and the entire experimental procedure were in accordance with *Codes for Experimental Use of Animals* of the Council of Agriculture, Taiwan, based on the *Animal Protection Law*, Taiwan, and were approved by the Institutional Animal Care and Use Committee of National Taiwan University.

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Surgical procedures

The rat was anesthetized initially with pentobarbital sodium (50 mg/kg, ip). Ketamine hydrochloride (50 mg/kg, im) was administered as necessary to maintain a proper anesthetic depth so that the animal had no flexor reflex throughout the surgery period. The rat was mounted on a stereotaxic apparatus. A midline incision was made over the skull. After retraction of the skin and cleaning of the soft tissue, small craniotomies were made for the placement of intracortical microelectrodes in the primary sensorimotor cortex (SmI) and the ACC.

Two microwire array electrodes were implanted in each rat, one in the SmI and the other in the ACC. The eight-channel microwire array electrode was built in-house. Detailed procedures of the construction of the array electrode were previously published (Tsai et al. 2003). Briefly, this is a linear array of stainless-steel wires individually insulated with Teflon (50 μ m OD). There are eight channels in an electrode, and the distance from the first channel to the last channel is about 2 mm. Two small longitudinal holes were opened in the right frontoparietal bone. One of the arrays was implanted in the tail region of the SmI (Chapin and Lin 1984). The coordinates of the SmI were 1–3 mm posterior to and 2–3 mm lateral to the bregma, and about 1 mm deep in the cortex. The receptive fields of the individual channels in the SmI were ascertained when the rat was still under anesthesia.

The other microelectrode array set was implanted in the ACC 1–3 mm anterior to and 0.3–0.6 mm lateral to the bregma, and 1–2 mm ventral to the surface of the cortex. This region covers the most-responsive and -relevant zones of the ACC reported in the literature by unit recording (Hsu and Shyu 1997; Yamamura et al. 1996), brain imaging (Tuor et al. 2000), activation (Calejesan et al. 2000; Johansen and Fields 2004), and inactivation (Johansen et al. 2001; Kung et al. 2003).

A stainless-steel screw (1 mm OD) for EEG recording was placed in the skull over the left SmI, 2.5 mm posterior and 2.5 mm lateral to the bregma. This was for the laser-heat-evoked potential recording, because in previous studies it was shown that the laser-heat-evoked potentials from the tail stimulation can be recorded bilaterally on both sides of the SmI (Shaw et al. 1999, 2001). The ground electrode consisted of stainless-steel screws located over the top of the cerebellum (mid-occipital bone). Several stainless-steel screws were placed in the frontal and parietal bones for anchoring purposes. Two pairs of three-stranded stainless-steel wires (793400, A-M systems) were inserted into the neck muscle and in the tail-flick muscle, respectively, to record their respective EMGs. The holes in the skull and the implanted electrodes were sealed and secured with dental cement. The rats were allowed to recover for ≥ 1 wk after surgery.

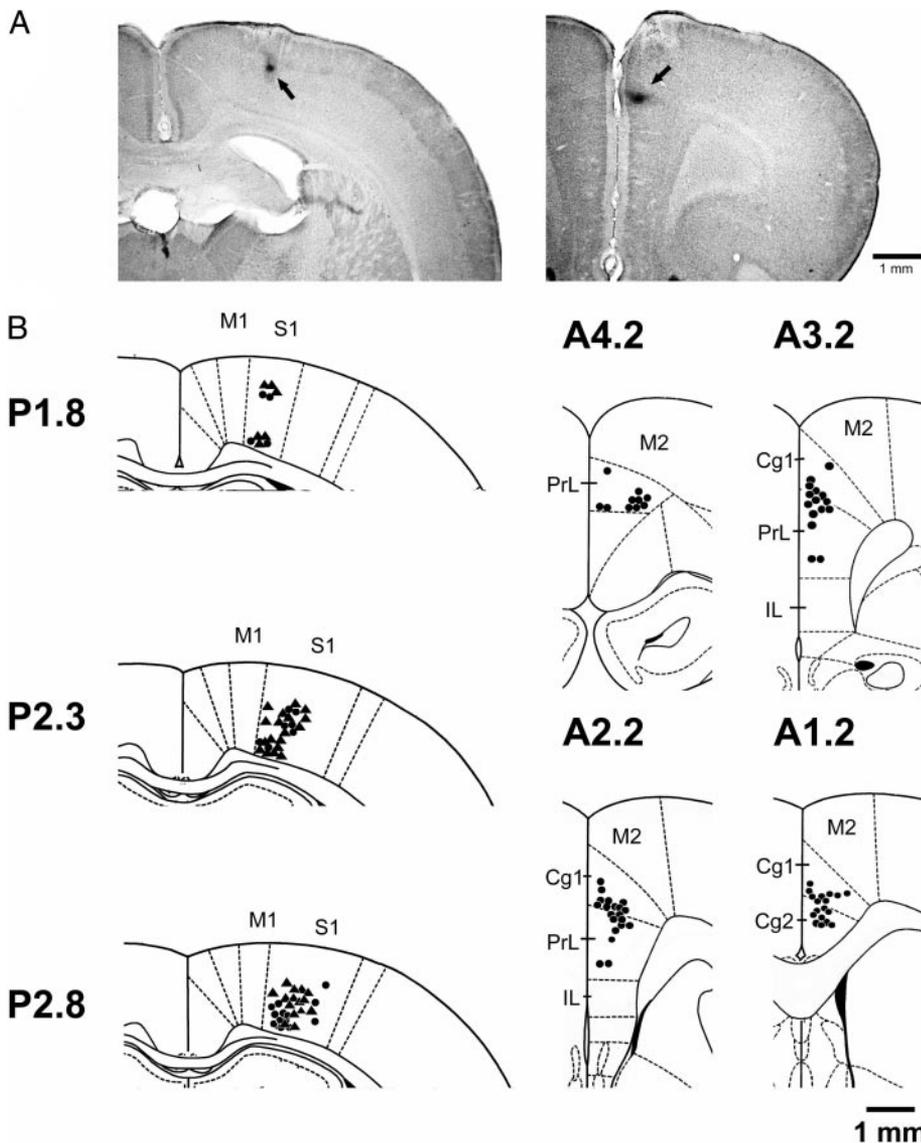


FIG. 1. Photomicrographs (*A*) and distribution (*B*) of recording sites in the primary sensorimotor cortex (SmI; *left*) and anterior cingulate cortex (ACC; *right*). Arrows in *A* point to iron deposits at the tips of the recording tract in the SmI (*left*) and the ACC (*right*), respectively. Because the 8-channel arrays were oriented parasagittally, only 1 of the 8 tracts can be seen in these 2 examples of coronal sections. Distribution of all recording sites was reconstructed onto maps in a standard stereotaxic atlas (Paxinos and Watson 1998) and is shown in *B*. Solid triangles represent recording sites that were responsive to light tactile stimuli of the tail of the rat, whereas solid dots are sites that were not responsive to light tactile stimuli. Numbers near the section indicate the anteroposterior level of the coronal section relative to the bregma in mm (A, anterior; P, posterior). Cg1, Cg2, cingulate cortex; IL, infralimbic cortex; M1, M2, motor cortex; PrL, prelimbic cortex; S1, primary somatosensory cortex.

Experimental protocol

Before the experiment, the animal was transferred to a test chamber for >2 h/day for 5 days to habituate it to the experimental environment. On the day of the recording, a period of >30 min was allowed for the rat to become familiar with the chamber. The test chamber had an open top to facilitate the connection of the headset cable to the implanted electrodes. It was 36 × 24 × 30 cm in width, depth, and height, respectively. During the test trial, rats could move about in the chamber while tethered to the recording wire. The stimulus was generated by a CO₂ laser (medical surgical laser, Tjing Ling 2, National Taiwan University; 10.6- μ m wavelength), operating in the TEM₀₀ mode (Gaussian distribution) (Yen et al. 1994). The radiant heat pulses were applied to the middle portion of the tail. The duration of the stimulation pulse was 15 ms. The output power was 8 W. The output energy was 120 mJ, which was about 1.5 times the maximal stimulus intensity for a tail flick response as determined in preliminary trials. The beam diameter was 3 mm (intentionally unfocused). To minimize tissue damage, sensitization, and habituation, stimuli were randomly applied to a local skin area 1 cm in length in the middle part of the tail. The interstimulus interval was longer than 10 s. ≥ 20 laser heat stimulations were made in each trial, and the jerky movements of the tail (tail flick) were noted. Each recording period lasted about 5 min.

Three doses (2.5, 5, and 10 mg/kg) of morphine were tested. The sequence of the dosages was random and different dosages were given intraperitoneally by a separation of ≥ 2 days. On each test day, laser irradiation trials were made before the morphine injection (to serve as a control) and every 60 min after the morphine injection for 4 h. After the completion of the three doses, the specificity of the morphine effect was assessed with the opiate antagonist, naloxone. Naloxone (8 mg/kg) was administered intraperitoneally 30 min before administration of 10 mg/kg morphine, and laser irradiation of the tail was performed every 60 min after morphine (naloxone + morphine). Naloxone hydrochloride was obtained from Research Biochemical International (Natick, MA), and the morphine sulfate was obtained from the Narcotics Bureau, Taipei, Taiwan.

Neuronal activity was recorded using a 32-channel Multi-channel Neuronal Acquisition Processor system (MNAP, Plexon, Dallas, TX). The electrical signals were passed from the headset to an amplifier and band-passed filtered (spike preamp filter: 0.5 ~ 8.8 kHz, gain: 10,000 ~ 20,000; EEG and EMG filters: 3 ~ 90 Hz, gain: 5,000; or 0.7 ~ 170 Hz, gain: 2,500). Real-time spike sorting was controlled by

SortClient (Plexon), and the sampling rate of individual channels was 40 kHz.

Two controls were performed to ascertain that at least a part of the short- and long-latency SmI and ACC neuronal responses were sensory in origin. The first control experiment used the same laser stimuli on anesthetized and paralyzed rats that had completed all of the above protocols. Three rats implanted with SmI and ACC microwire arrays were used. After a control set of stimuli consisting of 40 ~ 50 single pulses of 15-ms, 8-W laser-heat stimuli given to the middle part of the tail, the rat was anesthetized initially with 4% halothane. The femoral vein was cannulated, and a warm solution of 80 mg/kg α -chloralose (Sigma, St. Louis, MO) in saline was given intravenously. One to 2 ml of 2% gallamine triethiodide (Sigma) was given intravenously until the rat stopped breathing and artificial ventilation commenced. The ventilation volume and rate were regulated so that the maximal end-expiratory CO₂ concentration was maintained between 3.5 and 4.5%. The rectal temperature of the rat was monitored and feedback-regulated at 37.5°C with a thermal blanket (Harvard). The same set of laser-heat stimuli was used to stimulate the mid-tail of the rat. Supplementary doses of gallamine and chloralose were given as necessary.

The second control was a reanalysis of data similar to the control in our previous report on SmI units (Tsai et al. 2004). We noted that, with the 5-mg/kg dose of morphine, the rat flicked its tail about half of the time. These trials were divided into flick and no-flick groups and the perievent (laser-heat stimuli) histograms of the SmI and ACC units were separately analyzed. In this way, the effect of movement (tail flick) on the SmI and ACC responses to laser heat irradiation of the tail could be identified if it existed.

After the experiments were completed, electrical lesions (50 μ A, 30 s) were made on selected electrodes of the microelectrode array. The animal was perfused intracardially with saline followed by 10% formalin. In several rats, the positions of the electrodes were marked by 30 s of a 5- μ A positive current to deposit iron ions. The animals were perfused with 10% formalin containing 1% potassium ferrocyanide to display the iron deposition (Fig. 1A). Brains were removed and placed in the same perfusion fluid for 7 days. The frozen brain was serially sectioned at a 100- μ m thickness with a sliding microtome. Sections were stained with crystal violet. The positions of the electrode tips were ascertained with camera lucida drawings under a stereomicroscope. Figure 1B shows the distribution of all electrode tips in the SmI and ACC.

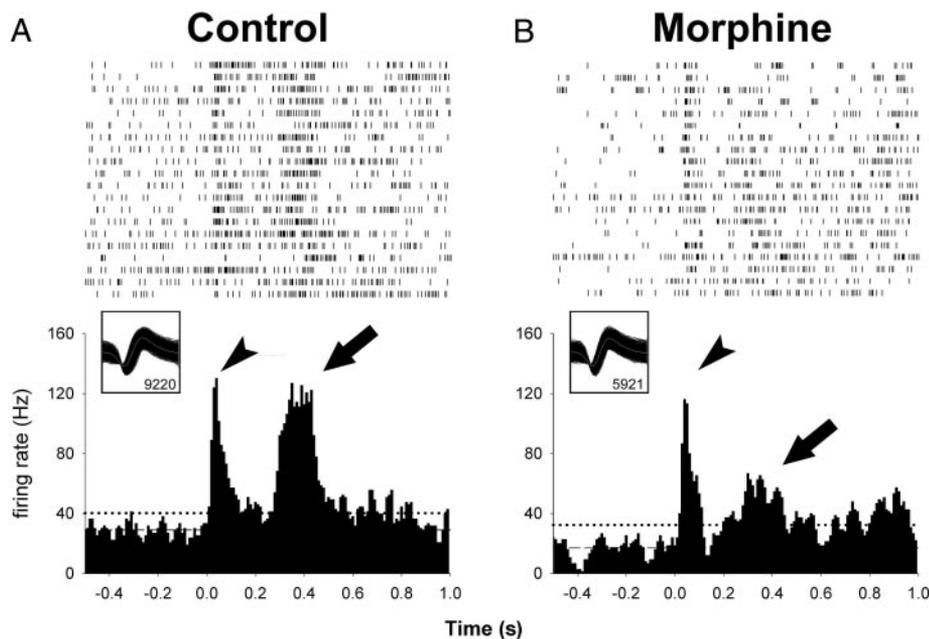


FIG. 2. Representative example of laser-heat-evoked SmI unit responses in the control state (A) and under the effect of morphine (B). At time 0, 20 trials of 8-W 15-ms laser-heat pulses were applied to the mid-tail of the rat. Dot raster plots are shown in the top panel, and average perievent histograms are shown in the bottom panel. Note that the long-latency responses (300 ~ 400 ms latency, arrows) in control trials were markedly decreased by an intraperitoneal injection of morphine sulfate (10 mg/kg). In contrast, the short-latency responses (50 ~ 100 ms latency, arrowheads) were largely unchanged. Dashed lines are the mean firing rate of the unit during the 500-ms periods before laser heat stimulation. Dotted lines are the 99% confidence line. Insets: superimposed wavelets (1-ms length) in the recording period of 358 and 314 s in the control and morphine-treated conditions, respectively. Number of superimposed spikes is indicated in the bottom right corner of each inset. Bin size: 10 ms.

Data analysis

The saved wavelet data were resorted using the software, Off-line Sorter, and analyzed using principal component analysis (PCA) and a template-matching algorithm (Plexon). Examples of SmI and ACC single units are shown in Figs. 2, 3, and 4. Spike activity was analyzed with the Neuroexplorer (Nex Technologies, Littleton, MA), Excel (Microsoft), and SigmaPlot (Jandel) programs. Peristimulation histograms were generated with a bin size of 10 ms. A "boxcar" filter (with a width of 3 bins) was used for postprocessing. Ensemble single-unit data (or single-unit data) were normalized as Z scores (Tsai et al. 2004). Briefly, the 0.5-s period before stimulation was used as the baseline period. The mean firing rate and SD of the 50 bins in this baseline period were calculated. All bin values were transformed to Z

scores according to the mean and SD of the baseline period. A 99% confidence level ($Z > 2.33$ or $Z < -2.33$) was used for identifying the responsive single-unit activity (or ensemble single-unit activity). All of the units recorded from channels in the SmI tail region and responsive units in the ACC were summed to obtain the ensemble activities. The SmI tail region was identified by the brisk responses restricted to the tail when the rat was under anesthesia during the surgery for implantation and the day the animal was killed.

The tail-flick ratio was calculated as the percent of tail-flicks in the total applied stimuli. The tail-flick ratios, and SmI and ACC neuronal responses were compared by one-way repeated-measures ANOVA followed by the Dunnett's method to compare values in the test trial with those in their respective control trials. Data are expressed as mean \pm SE where not specified.

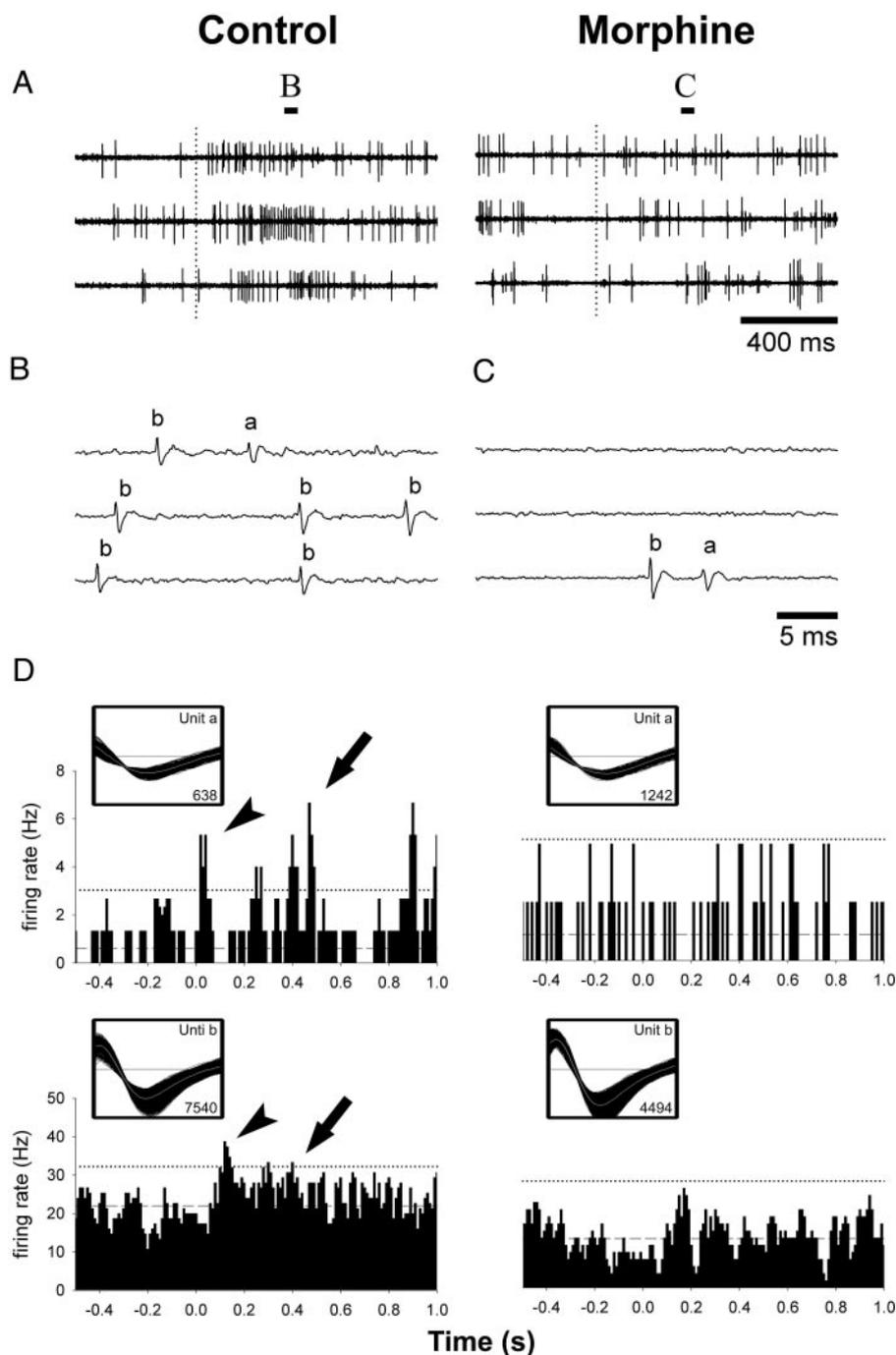


FIG. 3. Representative examples of original traces and average perievent histograms of laser-heat-evoked ACC unit responses in the control (*left*) and under the effect of morphine (*right*). The 2 ACC units were recorded by the same microwire sensor. At the time marked with vertical dotted lines in *A* and *time 0* in *D*, 25 8-W 15-ms laser-heat pulses were applied to the mid-tail of the rat. *B* and *C*: expanded portions taken from marked areas in *A*. Note the short- (arrowheads) and long-latency (arrows) responses in control trials in both units. These were all abolished with 10 mg/kg morphine sulfate (*ip*). For other conventions used, see the legend of Fig. 2.

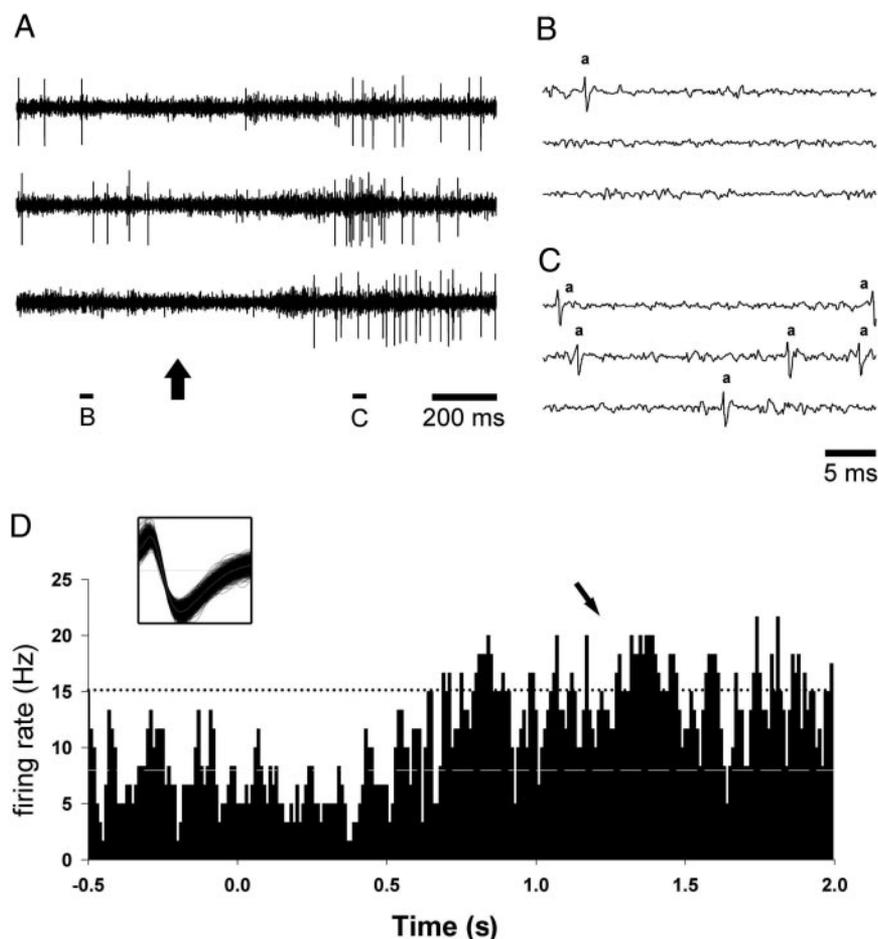


FIG. 4. An example of the very long latency, prolonged excitatory response to laser heat irradiation of the tail in an ACC unit. In the top panel are original traces in slow (A) and fast (B and C) time scales. Single laser-heat pulses (15 ms, 8 W) were administered to the middle part of the rat's tail at the time denoted with a thick arrow in A. B and C: expanded traces taken before (B) and after (C) stimulation. D: average perievent histogram from 20 laser pulses. Dashed line is average firing rate of the unit in the 500-ms prestimulation period, and dotted line is the 99% confidence line. Thin arrow points to the very long latency excitatory response of this ACC unit. Inset: superimposed 1-ms wavelets of all 2,815 spikes in the 236-s recording period. Bin size: 10 ms.

RESULTS

ACC and SmI responses to laser irradiation of the mid-tail

Supramaximal intensity of the CO₂ laser (120 mJ; Yen et al. 1994) was used in this study to obtain consistent and reproducible behavioral responses. At this intensity, consistent with previous studies (Shaw et al. 1999, 2001; Tsai et al. 2004), short- (LEP1) and long-latency (LEP2) evoked potentials were recorded from the EEG skull electrode in the rostral parietal region. The average LEP1 and LEP2 values of the eight rats were 67 ± 3 and 356 ± 7 ms, respectively (Table 1).

Seventy-four well-isolated single units were recorded in the tail region of the SmI of the eight rats. Consistent with our previous study (Tsai et al. 2004), most of these SmI tail units (88%) responded to laser heat irradiation of the mid-tail (Table 2). The latencies of their laser heat responses were bimodally distributed as shown in Fig. 5. In the same population of rats, 125 single units were recorded in the ACC. There were also short- and long-latency responses of numerous ACC units after laser heat irradiation of the mid-tail (Table 2). Accordingly, 150 ms was used as a defining line for short- (≤ 150 ms) and long-latency (>150 ms) cortical responses. Similar to units in

TABLE 1. Occurrence and latency of evoked potential, SmI, and ACC ensemble unit responses to laser-heat pulses applied to the mid-tail of rats

Morphine mg/ kg, ip	latency	Occurrence*			Latency, ms		
		LEP [†]	EUA _{SmI} [‡]	EUA _{ACC}	LEP	EUA _{SmI}	EUA _{ACC}
Control§	Short-	16/23	20/23	12/23	67 ± 3	52 ± 5	95 ± 11
	Long-	23/23	23/23	21/23	356 ± 7	368 ± 9	395 ± 23
2.5	Short-	6/7	7/7	4/7	61 ± 6	45 ± 3	85 ± 24
	Long-	5/7	7/7	6/7	385 ± 34	380 ± 29	417 ± 46
5	Short-	4/8	5/8	4/8	72 ± 17	39 ± 2	121 ± 18
	Long-	7/8	6/8	7/8	351 ± 33	373 ± 15	362 ± 43
10	Short-	5/8	5/8	1/8	78 ± 10	43 ± 4	103
	Long-	5/8	5/8	3/8	342 ± 46	359 ± 12	235 ± 54

Values are means \pm SE. §Includes control experiments for the three morphine doses. *The number of rats with this component/total number of rats tested. [†]Laser-evoked potential recorded from a skull electrode. [‡]Ensemble unit activity recorded with a microwire electrode in the tail region of the SmI or ACC. SmI, primary sensorimotor cortex; ACC, anterior cingulate cortex.

TABLE 2. Distribution of the number of different functional types of single-units with short- (*A*; ≤ 150 ms), long- (*C*; >150 and <600 ms), and very long-latency (*LL*; ≥ 600 ms) responses to laser heat irradiation (8 W, 15 ms) to the middle part of the tail in the tail region of the *SmI* and the *ACC*

	A+C+	A+C ⁰	A ⁰ C+	NR	LL	Inh	Total
<i>SmI</i>	25 (34%)	7 (10%)	32 (43%)	9 (12%)	0	1	74 (100%)
<i>ACC</i>	6 (5%)	5 (4%)	32 (26%)	61 (49%)	18 (14%)	3	125 (100%)

See Table 1 for other abbreviations. +, responsive; 0, not responsive; NR, not responsive at all latencies; Inh, inhibited.

the *SmI*, some units exhibited both short- and long-latency responses (6 units, 4.8%), whereas some had only a short-latency response (5 units, 4%), and more had only a long-latency response (32 units, 26%). Both short- and long-latency *ACC* unit responses peaked at longer latencies than did those of the *SmI* (Fig. 5). In addition, there were many *ACC* units that responded with initial latencies exceeding 600 ms. An example is shown in Fig. 4. The occurrence of different groups of units in the *SmI* and *ACC* as tested in the first control trial is summarized in Table 2. A χ^2 test of this 2×6 contingency table showed that the distribution of the types of neurons in the *SmI* and *ACC* significantly differed ($P < 0.001$).

For the anesthetized and paralyzed control, a representative result is shown in Fig. 6. In an awake and behaving condition, ensemble unit activities in the *SmI* showed very prominent short- and long-latency responses to laser-heat mid-tail stimulation. Less-prominent but still statistically significant short- and long-latency responses could be seen in the *ACC* ensemble unit response (Fig. 6, top left). Interestingly, under a wakeful condition, the *ACC* response was of a much-longer duration than that in the *SmI*, lasting >4 s. Under an anesthetized and paralyzed condition, ongoing cortical activities showed drastic decreases in both the *SmI* and *ACC*. The short- and long-latency laser-heat-evoked responses, however, became clearer. In this rat, the peak values in the *SmI* were 40 and 360 ms and in the *ACC* were 80 and 400 ms for the short- and long-latency laser-heat responses, respectively. Similar results were obtained in all three rats tested. Thus in an anesthetized condition and with no movement (paralyzed), the *SmI* and the *ACC* showed the same type of short- and long-latency responses to laser heat irradiation of the tail. On the other hand,

long-lasting and very long latency responses were no longer exhibited.

Morphine's effect on laser-heat-evoked *ACC* and *SmI* responses

The effects of an intraperitoneal injection of morphine on the laser-heat-evoked cortical responses were studied with 2.5, 5, and 10 mg/kg doses in six rats. In each trial, 20–30 laser pulses were shone onto the mid-tail of the rat. Behaviorally, the laser-heat-evoked tail flick was affected (Fig. 7A). In control trials, tail flicks were elicited by $98 \pm 1\%$ of stimuli. The tail-flick behavior was inhibited most effectively 60 min after morphine administration at doses of 5 and 10 mg/kg ($P < 0.01$). The suppressive effect of 10 mg/kg morphine on the tail flick ratio lasted >120 min ($P < 0.01$). Tonic neck muscle EMG activity under the three morphine doses showed no significant difference during the 5-s periods before laser heat application, indicating no severe sedating effect by morphine.

In a recent paper, we showed that short- and long-latency *SmI* unit responses were differentially affected by intraperitoneal morphine treatment (Tsai et al. 2004). Similar results were obtained in this study. Figure 2 shows an example of morphine's effect on *SmI* unit responses. In trials performed 60 min after morphine administration (Fig. 2B), it can be seen that the short-latency responses in the *SmI* were not affected, whereas the long-latency ones were suppressed.

Laser-heat stimulation of the mid-tail also elicited fast and slow responses in the *ACC* (Figs. 3, 5, 6, and 8). In contrast to the differential effects on fast and slow responses of the *SmI*, morphine treatment suppressed both fast and slow responses of the *ACC*. A single-unit example is shown in Fig. 3. Fast and

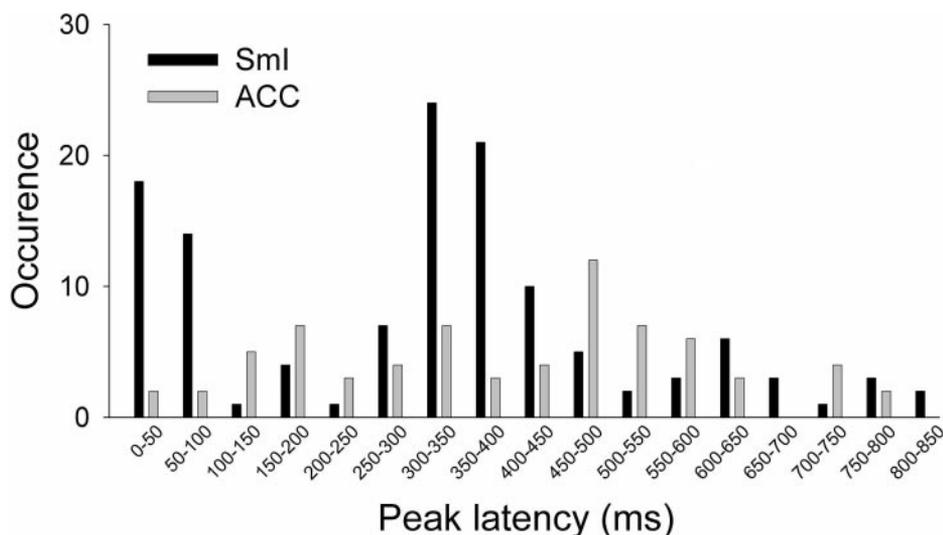


FIG. 5. Occurrence histograms of the latencies of all excitatory peaks of single units in the *SmI* (solid bars) and *ACC* (gray bars) in response to laser-heat stimulation of the mid-tail. Note the multi-modal distributions for both the *SmI* and *ACC*.

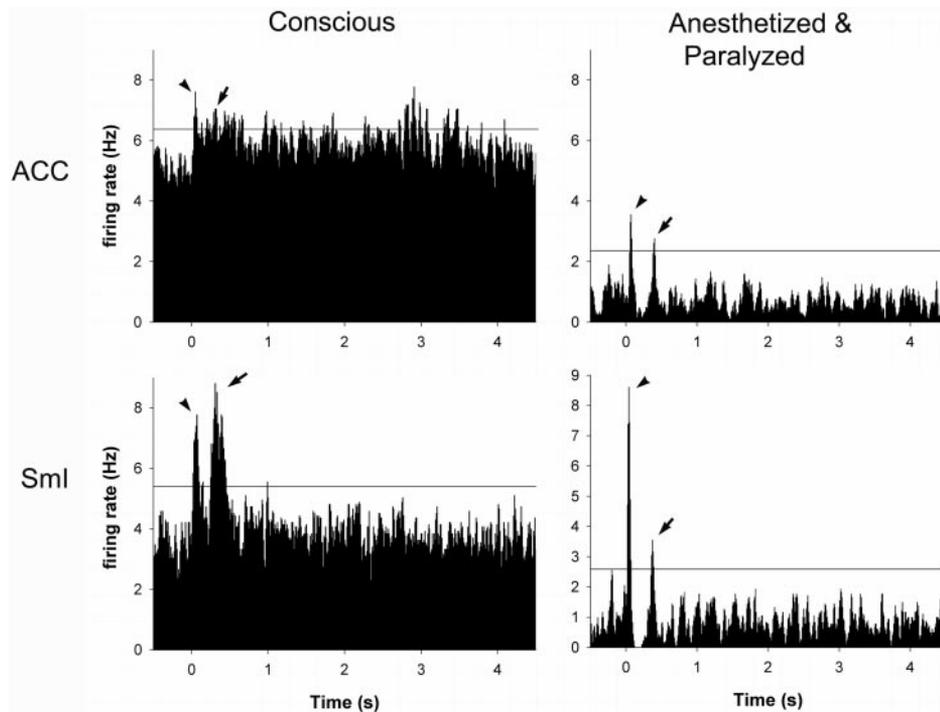


FIG. 6. Comparison of short- (arrowheads) and long-latency (arrows) laser-heat-evoked ACC (top) and SmI (bottom) ensemble single-unit responses in a representative rat that was conscious (left) or was chloralose-anesthetized, gallamine-paralyzed, and artificially ventilated (right). These are averaged perievent histograms of 50 (conscious) or 40 (anesthetized) laser-heat pulses of 15-ms duration and 8-W intensity applied to the middle part of the tail at time 0. Ensemble unit activity histograms are the linear average of 22 simultaneously recorded ACC and 9 SmI single units, respectively. Horizontal lines are the 99% confidence lines calculated with the ensemble activities during the 0.5-s period before laser heat stimulation. Note the heightened ACC activity for >4 s in the rat in the conscious, behaving state.

slow laser-heat-evoked responses of both units a and b in the ACC disappeared after an intraperitoneal injection of 10 mg/kg morphine. Ensemble SmI and ACC unit responses were statistically compared. Figure 8 shows results in one rat before and after treatment with 5 mg/kg morphine. Activities of a total of eight SmI units and seven ACC units were linearly summed and normalized to Z scores in this rat. In the control period, both SmI and ACC showed fast and slow ensemble responses. After a single dose of morphine, both fast and slow ACC responses drastically decreased, whereas in the SmI only the slow response was suppressed. The peak Z score values of the ensemble unit responses of the six rats are analyzed in Fig. 7, B and C. The SmI fast response did not significantly change with the three morphine doses (Fig. 7B), but the SmI slow response was significantly suppressed 60 min after the 5 mg/kg dose and for a longer duration after the 10 mg/kg dose (Fig. 7B). In sharp contrast, both the ACC fast and slow responses were very sensitive to morphine treatment. Both were significantly suppressed for ≥ 120 min by the 5 and 10 mg/kg doses of morphine (Fig. 7C). The fast ACC response was significantly suppressed for 240 min under the higher dose of 10 mg/kg (Fig. 7C).

As noted in Table 2, the ACC and the SmI contain many functional types of units. Whether different types of unit responses show different sensitivities to morphine treatment is analyzed in Table 3. Long-latency positive units were chosen because they are the group with the largest number of units. Within this group, there were two subtypes: the short-latency positive ($A^{+}C^{+}$) and the short-latency nonresponsive ($A^{0}C^{+}$) units. The SmI $A^{+}C^{+}$ units were significantly less affected by 10 mg/kg morphine than were the SmI $A^{0}C^{+}$ units ($P < 0.05$, χ^2 test). In turn, SmI $A^{0}C^{+}$ units were significantly less affected than were the ACC $A^{0}C^{+}$ units ($P < 0.05$, χ^2 test).

In the second control, we reanalyzed data with laser-heat irradiation of the tail of the rat under the 5 mg/kg dose of

morphine according to whether a flick of the tail occurred. A representative result is shown in Fig. 9. Note that in cases where no apparent movement occurred, prominent short- and long-latency SmI responses remained in the same latency range. In this rat, there were no obvious short-latency ACC responses following laser-heat stimulation of the mid-tail. The long-latency laser-heat-evoked ACC ensemble unit response did not noticeably differ regardless of whether or not the tail flicked. This analysis was carried out on eight rats. As in the case of the data reported in Table 1, short- and/or long-latency responses were observed in the SmI and the ACC in most but not all of the rats. Except for two cases in the SmI, all ACC data and the remaining SmI data exhibited the same type of response of the same magnitude when divided into flick and no-flick group of trials, be it short-latency only, long-latency only, short- and long-latency responses, or even no response. In the two SmI cases, one had a short-latency response in the flick trials, but a short- and long-latency response in the no-flick trials, whereas the other had a very strong combined response in the flick trials, but smaller yet still significant combined response in the no-flick trials. We concluded therefore that the bimodal type of laser-heat-evoked responses in the SmI and ACC were unlikely caused by secondary input from muscles and joints.

DISCUSSION

Single-unit responses to laser-heat stimulation of the middle part of the tail were recorded in the ACC and compared with those of the SmI using a multi-site, multiple single-unit recording technique. Many ACC neurons showed short- or long-latency responses. The ACC laser heat-evoked responses, however, had intriguing differences from those of the SmI. The effects of morphine on these neuronal responses were also tested. ACC unit responses to laser heat were affected to a greater extent by morphine than those in the SmI.

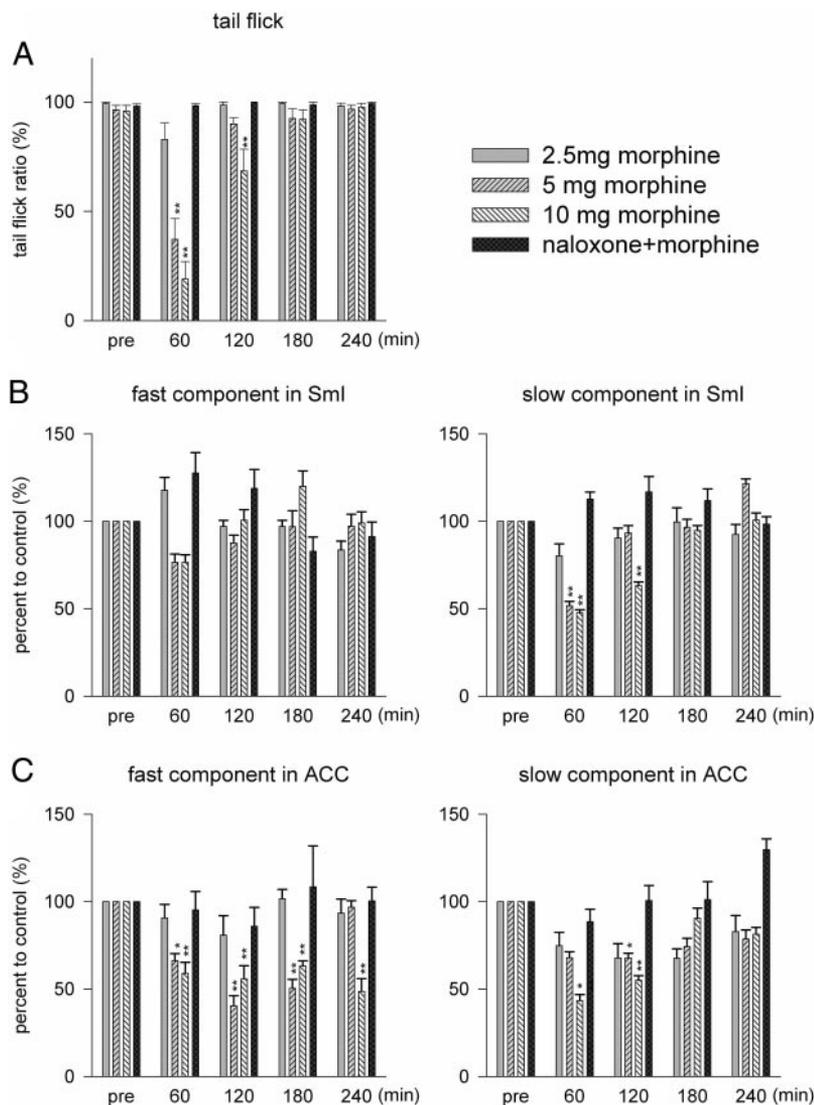


FIG. 7. Effect of an intraperitoneal injection of morphine sulfate on the tail flick behavior (A) and neuronal responses in the SmI (B) and ACC (C) of the rat. Morphine at 5 and 10 mg/kg significantly attenuated the tail flick and neuronal responses except for the fast component in the SmI. Attenuation by 10 mg/kg morphine was completely prevented by prior naloxone treatment (8 mg/kg, black column). In B and C, data in the experiment groups are percentages relative to that of control groups. * $P < 0.05$; ** $P < 0.01$.

In a recent paper (Tsai et al. 2004), we studied the responses of SmI units to laser-heat stimulation of the tail. As confirmed in this study, SmI units in the tail representation area respond to laser-heat pulses with either a short- and/or a long-latency response. When responses of many SmI units are summed, the ensemble activity consistently shows two peaks. These short- and the long-latency peaks match the LEP1- and LEP2-evoked potentials recorded by a screw over the skull of a rat, respectively. Single units were simultaneously recorded in the SmI and ACC in this study. About one-half of the ACC units also responded to laser stimulation of the tail. The most-prevalent types of responses observed in the ACC were also short- and long-latency responses (Table 2; Fig. 5). A new type of response pattern was found in the ACC, i.e., one with a very long-latency and long-lasting response (Figs. 4, 6, and 8; Table 2).

Outwardly they appeared similar, but when examined quantitatively, the short- and long-latency laser-heat-evoked responses in the ACC differed from those in the SmI. The percentage of ACC units that showed a short-latency response (defined as a latency < 150 ms) was only 8.8% (A^+C^0 and A^+C^+ combined in Table 2), in contrast to the 43% in the SmI.

The latency distribution of the short-latency response in the ACC ranged from 35 to 150 ms, with a median value of 125 ms, whereas the range in the SmI was from 30 to 100 ms, with a median value of 51 ms. The third difference in the short-latency response between the two areas was their sensitivity to morphine treatment. The SmI short-latency laser-heat-evoked response was not affected by morphine, even under a very large dose of 10 mg/kg. In contrast, the ACC short-latency response was very sensitive to morphine, and the inhibition was the strongest and the longest (cf. Fig. 7, B and C).

Percentage-wise, the ACC had far fewer units that responded in the long-latency range (defined as a latency exceeding 150 ms) to laser stimulation of the tail (38 of 125, 30%) than did the SmI (57 of 74, 77%). In other aspects, laser-heat-evoked long-latency responses in the SmI and ACC were more alike. Long-latency responses in both places had the highest peak, in the range of 300 ~ 350 ms, although the average latency in the SmI was consistently shorter than that in the ACC (Figs. 5 and 6). SmI and ACC long-latency responses were suppressed to a comparable degree by 5 and 10 mg/kg of morphine treatment for 120 min (Fig. 7, B and C).

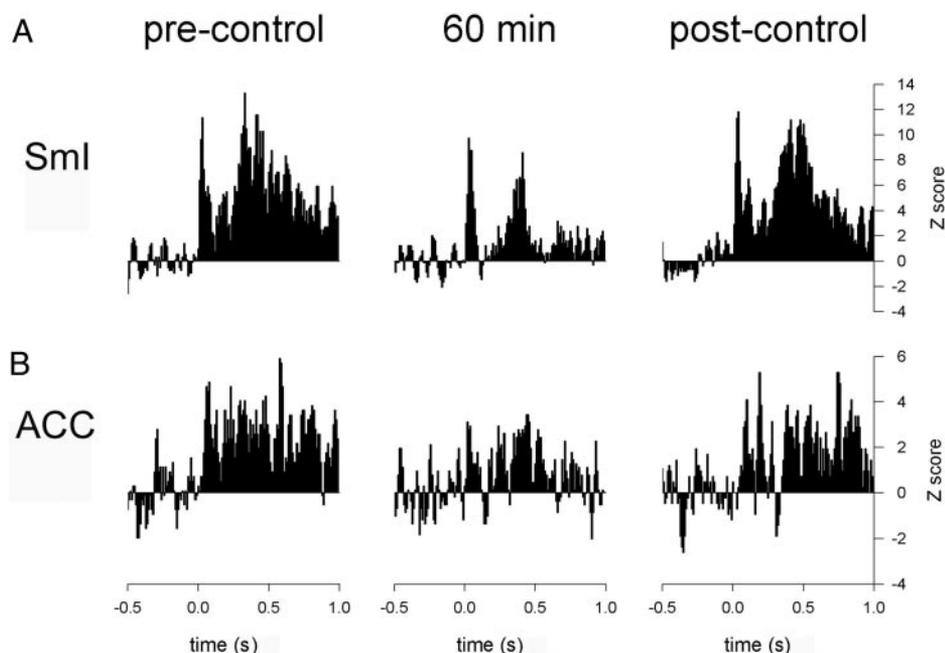


FIG. 8. Representative SmI and ACC ensemble unit responses to mid-tail laser-heat stimulation in 1 rat. These are perievent histograms of normalized ensemble activity of 8 SmI units (A) and 7 ACC units (B) averaged from 25 laser pulses given at *time 0*. *Left*: control experiment. *Middle*: responses obtained 60 min after a morphine injection (5 mg/kg, ip). *Right*: responses in the postcontrol experiment 24 h later. Note the bimodal responses in SmI and ACC controls. Long-latency response in the SmI and both short- and long-latency responses in the ACC were prone to morphine inhibition. Bin size: 10 ms.

Laser irradiation has been used as a specific noxious stimulus since the mid-1970s (Carmon et al. 1976, 1978). The 10.6- μm wavelength infrared radiation of the CO₂ laser selectively activates nociceptive receptors and generates a pure pain sensation, which is conveyed through both myelinated A- δ and unmyelinated C fibers to the cerebral cortex in humans (Bromm and Treede 1984, 1987). Two cortical laser-evoked potentials (LEP1 and LEP2) have been recorded from the cerebral cortex of the rat (Shaw et al. 1999, 2001). The conduction velocities of the peripheral fibers responsible for the activation of these LEPs have been estimated. Kalliomaki et al. (1993) stimulated different proximo-distal locations of

TABLE 3. Comparison of morphine's effect (10 mg/kg, ip) on the nociceptive responses of the major functional types of single units in the tail region of the SmI and the ACC

	Before Morphine		After Morphine	
	Type	Number and percentage	Type	Number and percentage
SmI	A ⁺ C ⁺	25 (100%)	A ⁺ C ⁺	14 (56%)
			A ⁺ C ⁰	4
	A ⁰ C ⁺	32 (100%)	A ⁰ C ⁺	4
			NR	3 (12%)
ACC	A ⁺ C ⁺	6 (100%)	A ⁺ C ⁺	6
			A ⁺ C ⁰	0
			A ⁰ C ⁺	12 (38%)
			NR	13 (41%)
ACC	A ⁰ C ⁺	32 (100%)	A ⁺ C ⁺	2 (33%)
			A ⁺ C ⁰	1
			A ⁰ C ⁺	2
			NR	1 (17%)
			A ⁺ C ⁺	2
			A ⁺ C ⁰	1
			A ⁰ C ⁺	6 (19%)
			NR	19 (59%)
			LL	4

+, responsive; 0, not responsive; A, short-latency (≤ 150 ms) response; C, long-latency (> 150 and < 600 ms) response; LL, very long-latency (≥ 600 ms) response; NR, not responsive at all latencies.

the hindpaw of halothane-anesthetized rats. A single long-latency cortical evoked potential was observed, and its peripheral conduction velocity was estimated to be 0.7 m/s. Shaw et al. (1999) stimulated different proximo-distal locations of the tail of conscious rats. LEP1 and LEP2 were observed, and their peripheral conduction velocities were estimated to be 22 and 0.7 m/s, respectively. Sun et al. (2003) analyzed the current source density of laser-evoked field potentials in the SmI of lightly anesthetized rats. Two major components were identified intracortically after laser stimulation of different proximo-distal locations of the hindpaw. Their conduction velocities from that research corresponded to the A- δ and C fiber ranges, respectively.

In this study, two consistent components of the ensemble unit responses to laser-heat irradiation of the tail were identified in both the SI and ACC (Figs. 2, 3, 5, 6, and 8). Although a direct study has yet to be performed for the ACC, our working hypothesis is that the two components in both regions arise primarily from A- δ and C fiber inputs, respectively. We showed here, in addition, that the response latencies in the ACC were longer than those in the SI. This is most likely due to the nature of synaptic transfer along the afferent pathways going to the two regions. The SI receives its major afferent input from the ventroposterolateral nucleus of the lateral thalamic pathway, in contrast to the ACC, which receives inputs from the medial thalamic pathway. The results in this brought forth a direct comparison of nociceptive signal transferring in the lateral and the medial thalamic pathways both in their synaptic efficacy and their modulation by morphine.

The emphasis of this study was on single units and regional ensemble unit study of cortical processing of nociceptive information. There have been many SI and ACC single-unit studies of their nociceptive information processing. Except for a very limited number of studies (Iwata et al. 1998; Kenshalo et al. 1988; Tsai et al. 2004; Wang et al. 2003), most studies were performed on the SI in anesthetized animals (Chudler et al. 1990; Iwata et al. 1990; Kalliomaki et al. 1993; Kenshalo

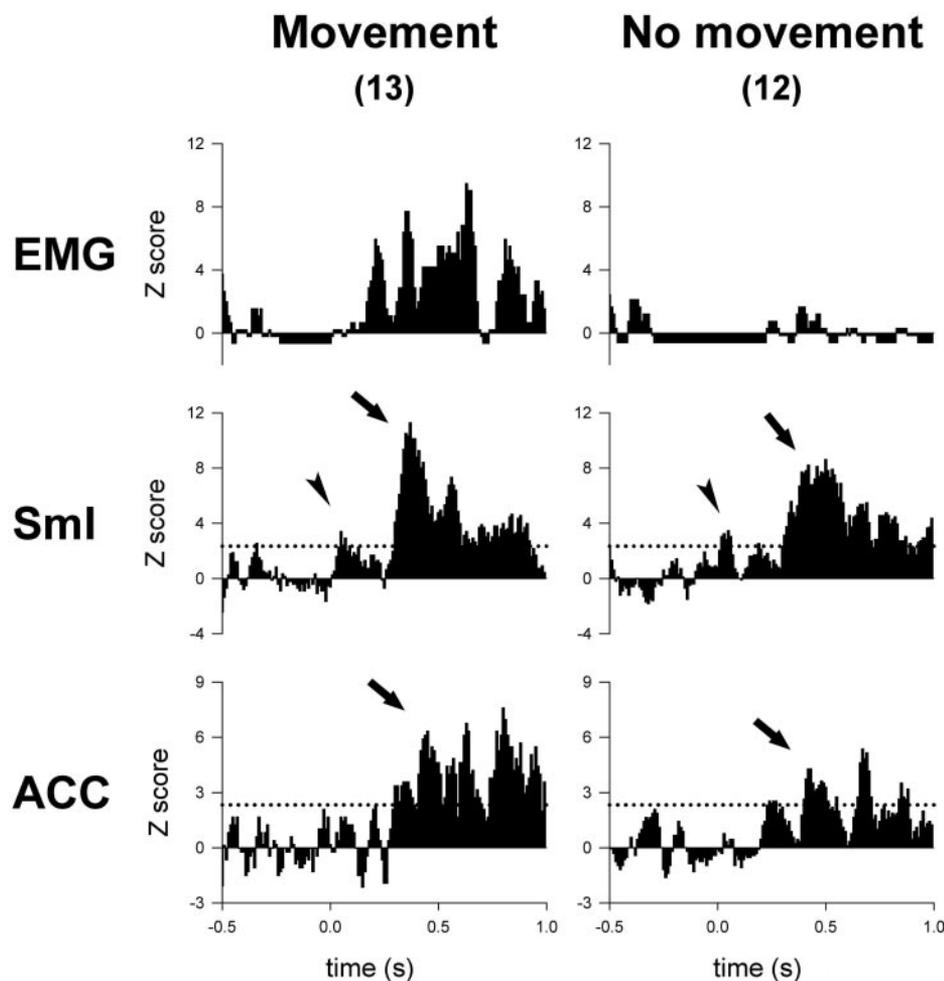


FIG. 9. An example showing short-latency (arrowheads) and/or long-latency (arrows) ensemble unit responses in the Sml (16 units, middle) and the ACC (16 units, bottom) analyzed according to whether a tail flick occurred (13 trials, movement) or did not occur (12 trials, no movement) after 8-W, 15-ms laser-heat irradiation of the mid-tail. EMG (top) was recorded by sensors implanted in the tail flick muscle. Note that the same type of ensemble unit responses occurred with or without accompanying tail flick.

and Isensee 1983; Kenshalo et al. 2000; Lamour et al. 1982, 1983a,b; Matsumoto et al. 1989) and in the ACC (Sikes and Vogt 1992; Vogt and Sikes 2000; Yamamura et al. 1996; Zhang et al. 2004). SI nociceptive neurons have relatively smaller receptive fields. The activities of these neurons show good coding ability for stimulation intensity. In contrast, the receptive fields of nociceptive neurons in the ACC are larger and usually bilateral. Using the ensemble unit-recording technique, this study adds quantitative information about the nociceptive processing of SI and ACC neurons. In addition, by simultaneously recording as many as 20 single units in the SI and ACC in the same animal, populations of SI and ACC neuronal activities could be compared. Although individual single units showed variable response patterns, collectively, fairly robust responses to noxious laser-heat were observed in both the SI and ACC. Ensemble activity of the SI showed consistently faster and stronger short- and long-latency responses than those of the ACC. An unexpected finding revealed by this analysis was a long-duration response of the ACC ensemble activity (Figs. 4 and 6), which could last for more than a few seconds. This is a pattern similar to the result obtained in the ACC with magnetoencephalograms of human subjects in response to single shots of laser-heat applied to the dorsum of the hand (Ploner et al. 2002). How this slower-onset, longer-duration response in the ACC contributes to affective, attentional, or cognitive aspects of pain function awaits further experimentation.

Another set of data of this study relates to the effect of morphine (ip) on the laser-heat-evoked cortical responses. Interestingly, the ACC showed higher sensitivity to morphine treatment than did the SI (Fig. 7; Table 3). This is consistent with the biochemical findings of a higher density of opiate receptors in the limbic cortex, including the ACC (Jones et al. 1991; Lewis et al. 1983; Mansour et al. 1986, 1987). It has been shown that morphine at lower doses is effective in reducing pain emotion and that a higher dose is required to decrease the pain sensation (Price et al. 1985). Thus it may be possible to dissect different components of pain pharmacologically by the use of morphine. By showing that the ACC ensemble unit response to noxious heat stimuli is more sensitive to morphine treatment, our data added cellular evidence in support of the important role of the ACC in the emotional function of pain (Hamner et al. 1999; Johansen et al. 2001; Kung et al. 2003).

In summary, by using a modified tail-flick model in conscious rats, this study showed many interesting differences in laser-heat-evoked responses in the Sml and ACC. Differential sensitivities of these nociceptive responses to morphine treatment were also quantified. These data may be useful in dissecting the mechanisms of different aspects of pain function.

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GRANTS

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