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Nociceptive Neurons in Area 24 of Rabbit Cingulate Cortex

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SUMMARY AND CONCLUSIONS

1. Single-unit responses in area 24 of cingulate cortex were examined in halothane-anesthetized rabbits during stimulation of the skin with transcutaneous electrical (TCES, 3-10 mA), mechanical (smooth or serrated forceps to the dorsal body surface or graded pressures of 100–1,500 g to the stabilized ear) and thermal (>25°C) stimulation.

2. Of 542 units tested in cingulate cortex, 150 responded to noxious TCES (≥6 mA), 93 of 221 units tested responded to noxious mechanical (serrated forceps) and 9 of 47 units tested responded to noxious heat (>43°C) stimuli. Twenty-five percent of the units that responded to noxious mechanical stimuli also responded to noxious heat stimuli. The only innocuous stimulus that evoked activity in cingulate cortex was a "tap" to the skin and this was effective for 11 of 14 tested units.

3. In 74 units that produced excitatory responses to TCES of the contralateral ear, response latency was 166 ± 11.3 (SE) ms and response duration was 519 ± 52.1 ms.

4. Twenty of the 150 units that responded to noxious TCES were initially inhibited. These responses were usually <1 s in duration (17 of 20 units), whereas responses in the other 3 lasted for over 20 s.

5. Most units had broad receptive fields, because noxious mechanical stimuli anywhere on the dorsal surface of the rabbits, including the face and ears, evoked responses. A small number of units for which the entire body surface was tested (3 of 15 units) had receptive fields limited to the ears, rostral back, and forepaws.

6. Fifteen of 33 units tested had no preferential responses to noxious TCES of the ipsilateral and contralateral ears. Of the remaining units, 10 had a greater response to contralateral and 8 had a greater response to ipsilateral stimuli.

7. The locations of 186 units were histologically verified. Most nociceptive cingulate units were in dorsal area 24b in layers III (n = 35), II (n = 13), or V (n = 9).

8. Cortical knifecut lesions were made in five rabbits to determine if the responses in area 24 were dependent on lateral or posterior cortical inputs. These lesions did not alter the percentage of units driven by noxious stimuli nor response latency.

9. Injections of lidocaine were made into medial parts of the thalamus in six animals and injection and recording sites analyzed histologically. The excitatory responses evoked in 12 cortical units with noxious stimuli were virtually eliminated with lidocaine injections that were in the parafascicular nucleus or as far rostral as the submedial nucleus.

10. The broad receptive fields and high thresholds of the area 24 neurons were similar to responses described previously in the medial and intralaminar thalamus in several species. Because area 24 receives substantial input from these thalamic nuclei and the cingulate cortical responses could be blocked with thalamic lidocaine injections, thalamic projections may mediate the cingulate responses to noxious stimuli.

11. The presence of nociceptive neurons in area 24b clarifies previous observations that area 24 *1*) is crucial for avoidance learning that employs noxious footshock as the unconditional stimulus, *2*) has unit activity that is highly correlated with heart rate in classical conditioning paradigms, and *3*) is a suitable neurosurgical target for the alleviation of pain in patients with chronic diseases.

INTRODUCTION

There is a growing body of evidence that anterior cingulate cortex may be involved in pain sensation and/or responses to noxious stimuli. Positron emission tomography findings suggest that there is elevated blood flow in anterior cingulate cortex during the application of 46–49°C heat stimuli that are reported by subjects as painful but tolerable (Jones et al. 1991; Talbot et al. 1991). It is unlikely that activation of cingulate cortex is due to arousal or stress because conditioning sessions reduce subject stress but not cortical activation to noxious stimuli. Also, Reiman et al. (1989) have shown that "anticipatory anxiety" associated with painful electric shock elevates blood flow bilaterally in temporal pole cortex, but not in cingulate cortex.

There is neurosurgical evidence for the involvement of anterior cingulate cortex in pain perception. The cingulotomy procedure involves ablating part of the white matter that underlies anterior cingulate cortex in a select patient population. This operation can alleviate a patient's affective responses to noxious stimuli, like those produced by chronic cancer, whereas preserving the ability to localize such stimuli (Amromin et al. 1975; Ballantine et al. 1987; Foltz and White 1962; Turnbull 1972; White and Sweet 1969).

Studies of experimental animals also support the putative role of cingulate cortex in pain sensation and/or responses. A consistent behavioral finding in cingulectomized animals is a disruption in active avoidance learning, i.e., learning that involves avoidance of noxious footshock (Gabriel et al. 1991a; Lubar 1964; Lubar and Perachio 1965; Peretz 1960; Thomas and Slotnick 1963). Furthermore, lidocaine injections into the cingulum bundle reduce behavioral measures of pain after Formalin injections into rat forepaws (Vaccarino and Melzack 1989). This latter observation reflects disruption of cingulate function and/or activity in connections between other cortical areas.

It is possible that there is a direct route for nociceptor-generated activity to cingulate cortex via its connections with the medial and intralaminar thalamic nuclei. The reuniens, parafascicular, centrolateral, submedial, and ventromedial nuclei receive direct spinal cord inputs including those from laminae I and V (Carstens and Trevino 1978;

Craig and Burton 1981, 1985; Giesler et al. 1979; Granum 1986; Menetrey et al. 1984; Willis et al. 1979). Neurons in the centrolateral, parafascicular, and submedial nuclei respond to noxious thermal and mechanical stimuli and have almost no somatotopic organization (Casey 1966; Dong et al. 1978; Dostrovsky and Guilbaud 1988; Miletic and Coffield 1989; Peschanski et al. 1981), and lesions in the centrum medianum-parafascicular complex alter escape responses to noxious electrical stimulation of the tooth pulp (Kaelber et al. 1975). In addition, the reuniens, parafascicular, centrolateral, and ventromedial thalamic nuclei project to anterior cingulate cortex in all species studied (Baleydier and Mauguiere 1980; Cunningham and Levay 1986; Macchi et al. 1977; Musil and Olsen 1988; Robertson and Kaitz 1981; Vogt et al. 1979, 1987), whereas the ventral paralaminar region of the ventrobasal nucleus projects to anterior cingulate cortex in the cat (Yasui et al. 1988), and the submedial nucleus projects to this cortex in the rabbit (Vogt et al. 1992). Thus it is possible that nociceptive activity is transmitted to cingulate cortex via the medial and intralaminar thalamic nuclei.

The strategy of the present study was guided by the following considerations. There is a large body of data regarding plasticities in the discharges of rabbit cingulate neurons throughout the course of classical and instrumental learning that employs noxious shock as the unconditional stimulus (e.g., Gabriel et al. 1980, 1991a, b; Gibbs and Powell 1991). In addition, our recent anatomic and opioid receptor localization studies have employed the rabbit brain because it has a highly differentiated cingulate cortex and associated limbic thalamic nuclei (Vogt et al. 1986, 1992; Vogt and Sikes 1990). Because there are no studies available that analyze responses of cingulate neurons to noxious stimuli, the present study was undertaken in the rabbit to *1*) determine whether or not neurons in cingulate cortex respond to noxious stimuli, *2*) assess the receptive field properties of these neurons, and *3*) identify the source of such responses with cortical knifecut lesions and thalamic lidocaine injections.

METHODS

Surgical procedures

Forty-eight male, Dutch-belted rabbits (2–5 kg) were used in this study. Each animal was initially anesthetized with an intramuscular injection of a mixture of ketamine and xylazine (35 mg ketamine and 5 mg xylazine/kg; White and Holmes 1976). After a surgical level of anesthesia was obtained, a tracheostomy was performed and then 2–3% halothane with a mixture of 75% NO-25% O_2 was administered to maintain surgical levels of anesthesia. Heart and respiration rate monitors and a rectal temperature probe were attached to the rabbit and monitored throughout the surgery and recording session. The animal was wrapped in a thermostatically controlled thermal blanket to maintain body temperature at 37°C.

The skull was stabilized for recording by a stainless steel bar. To attach the bar, the skin and periosteum over most of the frontal and parietal bones was removed. Small stainless steel screws were inserted into the skull at several points, and the steel supporting bar was cemented to the screws with fast drying dental acrylic. A well was formed over the recording area with the acrylic. This well was filled with warm 0.9% NaCl during the recording session. The bar was attached to a stereotaxic frame and the head was positioned so that the skull was approximately level. A small opening (~2 × 3 mm) was drilled through the skull to allow access to anterior cingulate cortex. The openings were made between the stereotaxic coordinates of 2–6 mm in the anteroposterior plane and 0-2 mm lateral to the midline. Because this opening was small and the dura mater intact, very little pulsation of the brain occurred.

After the surgery was completed, the halothane was reduced to 0.5%. Heart and respiration rate were regularly monitored throughout the experiment to ensure that the animals were consistently anesthetized. Recordings began ~1 or 2 h after the surgery. Although cortical activity was reduced by the anesthesia, responses to noxious stimuli were consistently recorded in cingulate cortex. Because these animals had intact neuromuscular transmission, corneal and withdrawal reflexes could be regularly assessed during the experiment as an additional check of anesthetic level. The percentage of halothane was maintained at a level where reflexes were nearly absent or very weak and sluggish. At this level, there were no signs of arousal such as increased respiration or heart rate, or coordinated movements, but robust neuronal activity in the cortex was reliably obtained. Deeper levels of anesthesia severely diminished cortical responses and spontaneous activity.

Recording techniques

Lacquer-insulated, 2–4 MΩ tungsten electrodes were used to record single-unit activity in cingulate cortex and the thalamus. These electrodes had 2 μm of exposed tip and an abrupt taper to allow penetration of the dura. Each electrode was stereotaxically targeted with bregma, the dural surface, and the midline as reference points. Penetrations were made at ~0.5-mm steps within the skull opening and were placed to avoid the numerous blood vessels near the midline. Extracellular potentials were amplified and filtered with a Grass P15 amplifier (Grass Instruments, Quincy, MA), and the resulting signal was monitored with a digital oscilloscope and an audio monitor and converted into logic pulses with a window discriminator. The electrodes were advanced until the spontaneous activity of a single unit could be accurately discriminated by amplitude. Only these single-unit isolations were tested for responses to noxious and innocuous stimuli. There were no instances in which "silent" units were observed to respond to these stimuli. The output of the window discriminator was sampled at 1-ms intervals and displayed in real time as rasters and peristimulus histograms with the MI^2 data acquisition hardware and software (Modular Instruments, Southeastern, PA). The raw data were stored on disk for off-line statistical analysis.

Stimulation techniques

Three forms of peripheral stimulation were used to test isolated units: transcutaneous electrical (TCES), mechanical, and thermal stimulation. In early recording sessions, qualitative mechanical stimulation, as described below, was used to test the responses of isolated cells to noxious and innocuous stimulation. Because this technique may damage tissue, the number of tests that could be performed during a single session was limited. In subsequent experiments, TCES was used as a search stimulus, because stimuli over 5 mA reliably evoked pain in the conscious investigator and caused no tissue damage when applied repeatedly to the ear. Isolated units that did not respond to noxious TCES were often tested with qualitative mechanical stimulation applied to the dorsal body surface. The TCES was delivered through a constant current isolation unit (PSIU-6, Grass Instruments) to bipolar ear-clip electrodes (modified Grass reference electrodes) that were applied with conductive paste to a shaved region at the tip of each ear. The conductive paste was periodically moistened with saline or re-

placed during the experiment. TCES search stimuli consisted of 1-ms pulses at 6–10 mA configured as either single pulses or in trains of stimuli at 100 Hz lasting 20–50 ms. The interstimulus interval was usually 10 s. Because the conscious investigator characterizes these stimuli as painful when they were >5 mA, stimuli of 6–10 mA were employed to ensure that nociceptors were activated. Villanueva et al. (1989) observed that neurons in the reticular formation code for the number of Aδ and C fibers activated with TCES and that maximal responses to C fiber stimulation occurred after 12-mA pulses. Units that responded to noxious levels of TCES were often tested with low-intensity TCES or light stroking, brushing, or pressure to establish whether they also responded to lower amplitude stimuli.

About one-half of the isolated units were tested with mechanical stimulation applied to the dorsal surface of the rabbit. Most of these cells were selected by their responses to noxious TCES, but 90 units were classified by their responses to noxious mechanical stimulation alone. Most of the ventral surface was inaccessible in this preparation. For most cells, a qualitative determination of mechanical responses and the extent of the unit's receptive fields was used. Artist's brushes, blunt and sharp probes, blunt and sharp surgical forceps with a 3 × 7 mm surface, and innocuous TCES were applied to the body surface of the rabbit. Some neurons were tested with "tap" stimuli. The ear or other body area was lightly tapped with a blunt probe that was calibrated to have a maximal force of 150 g. The contact of the probe to the tissue was short, i.e., ~0.1 s, and the velocity of the probe at contact was much higher than the other forms of mechanical stimuli. When applied to the experimenters, these stimuli were innocuous.

For some cells, calibrated mechanical stimuli were applied to the stabilized ear, which is an ideal surface for the application of controlled noxious stimuli. Shea and Perl (1985) evaluated C fiber activity in a cutaneous nerve of the ear of urethane-anesthetized rabbits and showed that most were nociceptors and that all responded to noxious pressure. In the present cases, the ears were fixed to rigid platforms and calibrated probes were used that produced forces from 100 to 1500 g in 100-g steps. The probes had a tip diameter of 1.5 mm and were inserted into a sheath containing a spring. Each probe was calibrated by measuring the distance that a reference point moved as known forces were applied to the tip. The onset of the stimulus was synchronized to the data acquisition system by a high impedance circuit that emitted a logic pulse when the stylus contacted the ear. The investigator's pain threshold for this device was 300–400 g, and ear withdrawal was evoked with similar intensities of stimuli in the conscious rabbit. The stimuli were presented in random order and lasted from 0.1 to 10 s. Intervals of 20–60 s elapsed between the stimuli. Because intense mechanical stimulation can produce tissue damage, the points of application were marked with a felt pen and stimuli were separated by ≥1 cm. Only one or two graded series were tested in a single animal.

Thermal stimuli were applied with a thermal probe (Analytic Technology, Redmond, WA) that had a 3-mm tip diam. This unit was capable of generating temperatures from room temperature to 65°C. The temperature was determined by a thermistor that was attached to the tip and had a linear relationship between voltage output and probe temperature. To test a cell, the probe was heated to ~50°C and then applied several times to different parts of the ear. If a response was obtained, then a graded series of tests were made either by applying the probe to the ear at room temperature and letting it heat continuously to 55°C or by applying the stimulus for 3 s at ~5–10°C steps of temperature. The order of the stimuli intensities was varied from test to test and interstimulus intervals were 30–60 s. Because the higher levels of thermal stimulation could damage tissue, the application points were marked and separated by ≥1 cm, and only one or two series were conducted in a single animal.

Cortical ablation and thalamic lidocaine injection techniques

In five rabbits, knifecut lesions were made in the cortex to separate the cingulate cortex from adjacent, lateral, and posterior cortical regions. After recording to establish the location of nociceptive units, the opening in the skull was enlarged. In two animals, a scalpel cut was made 2 mm lateral to the midline and 5 mm deep, extending from 2 to 6 mm anterior to bregma. In three other animals, these lateral lesions were extended to posterior levels of cortex and included the cingulum bundle. Bleeding was controlled with gelfoam, and the brain was allowed to recover for ≥1 h before unit recordings were undertaken. Lesions that removed inputs from rostral cingulate and some orbitofrontal areas were not employed because thalamic afferents to area 24 course through the cingulum bundle at these rostral levels.

In six rabbits, lidocaine injections were made into the thalamus. In these animals, exploratory probes were made into the thalamus at the sterotaxic coordinates of the parafascicular nucleus until responses to noxious stimuli were evoked. Then a recording electrode cemented to a Hamilton syringe was lowered to this location. The tip of the electrode extended 1 mm beyond the tip of the cannula. Units with responses to noxious TCES were again located, and the assembly was lowered 1 mm to position the cannula that contained 2% lidocaine or 0.9% NaCl. After a nociceptive unit was located in cingulate cortex and its responses were characterized, an injection of 1–2 μl of lidocaine was made into the thalamus for 30–60 s. The effectiveness of these injections was observed by monitoring the spontaneous activity of single- or multiple-unit activity at the thalamic recording electrode. Spontaneous activity in the thalamus was abolished by the injection but recovered with a time course approximately equal to the recovery of responses in cingulate cortex. Because the thalamic recording electrode was within 1 mm of the cannula tip, the effective radius of the lidocaine blockade was ≥1 mm from the cannula tip. The opening of the cannula always faced toward the medial part of the thalamus.

Data analysis

Determination of a response to noxious stimulation was initially done qualitatively by listening to the response on the audio monitor and by observing the raster and histogram display by the MI[2] software. Generally, only the data from units with a response that exceeded twice the baseline activity to at least one of the stimulation modes were saved for quantitative analysis. The responses were plotted as peristimulus histograms when the stimuli were presented every 10 s or as a plot of the average frequency within 1-s bins.

A quantitative analysis of the response latency and duration was performed on responses to TCES with ≥10 sweeps in the histogram. The mean frequency of "moving window" samples from the histogram were statistically compared with the mean baseline response with the one sample t test. Each sample contained 10 bins, and each bin in the sample was 10 ms in duration. The onset of the response was the center of the first window with a mean that differed at the 95% confidence level from the baseline mean for two consecutive windows. Conversely, the offset was the bin at which the response no longer differed significantly from the baseline mean for two consecutive windows. It should be mentioned that this method was more reliable than the intuitive approach of establishing a 95% confidence interval around the baseline mean, because of the extremely low spontaneous activity encountered in the cingulate cortex units. Measurement of latency and duration made with this method corresponded well with qualitative estimates of these parameters from histograms with an obvious onset and offset and provided an unbiased and repeatable method for estimating response characteristics that were more variable.

This same method was used to compare responses with TCES in ipsilateral and contralateral ears. The strength of the response to TCES was calculated as the mean firing frequency during the response minus the baseline mean. The difference in the responses was tested with the two sample t test. TCES was used for this determination because of the control and repeatability of this stimulus. The order of the presentation was varied.

Responses to graded series of mechanical stimuli were assessed with a one-way analysis of variance (ANOVA). The Newman-Keuls test for multiple comparisons was used to evaluate differences in response amplitudes for individual levels of stimulation, i.e., at each force level of stimulation.

Histological analysis

At the end of each experiment, small electrolytic lesions (−50 μA for 10 s) were placed with the recording electrode at the end of the penetration or at the location of a responsive unit. Rabbits were never allowed to regain consciousness. Each animal was given a high dose of ketamine-xylazine and then perfused with 0.9% NaCl followed by 10% Formalin. The brains were removed to the same fixative for several days and then transferred to a cryoprotectant (10% sucrose and 10% glycerol) until they sank. Frozen sections 40 μm thick were cut in the coronal plane and all sections saved through the recording sites in the cingulate cortex and, in the lidocaine injection cases, in the thalamus. The sections were mounted and stained with thionin.

Reconstruction of the anteroposterior location of units was made by locating the lesion relative to the genu of the corpus callosum. With the stereotaxic coordinates of the lesion and the isolated units, the location of all units relative to the genu could be determined for individual animals and then combined to give an overall distribution of the responsive and nonresponsive units. In some cases, the lesion could not be clearly identified, although electrode tracks could usually be found. In these cases the stereotaxic coordinates, adjusted for the location of the identified tracks in the same case, were used to locate the units.

The vertical orientation of the anterior cingulate cortex complicates laminar reconstruction, because all penetrations are oblique. For this analysis, only units along the tracks marked with a lesion were considered, because the depth and orientation of the track could be accurately determined. Each section at the lesion was drawn at ×30 magnification, and the path of the penetration and location of each cortical layer was identified according to criteria for area 24 (Vogt et al. 1986). Areas 24b and 8 both have occasional neuronal aggregates in layer II, there is no layer IV in either area and they have a relatively constant layer Va of large pyramidal neurons. These areas can be distinguished with the following criteria. Neuron density in layers II and III of area 8 is much reduced in comparison with that in area 24b. There is a very thin layer Vb in area 24b that has some neurons, whereas in area 8 this layer is much broader and contains few neurons. Finally, layers VIa and VIb are cell sparse in area 8.

The location of units along each track was plotted with their coordinates relative to the lesion. Shrinkage effects were removed by comparing the stereotaxic distance from the first sign of multiunit activity with the histological distance from the top of layer II to the lesion. Significant differences in layer distribution were tested with χ^2 analysis.

RESULTS

Responses to noxious stimuli

Table 1 shows the categories of responses for units in anterior cingulate cortex. Of the 643 units isolated, 542 were tested with TCES and 150 had responses to noxious intensities ≥6 mA. Of the 221 units that were tested with noxious mechanical stimuli, i.e., pinches with serrated forceps, 93 units responded. Of these 93 units, 79 were tested with noxious TCES, and all but 2 had significant responses. Twelve cells that responded to the noxious TCES search stimulation could not be driven by any mechanical stimulation. Forty-seven units with responses to TCES were tested with noxious heat stimulation over 43°C, and 9 units responded. No units responded to innocuous mechanical stimulation such as light stroking of the skin or pressures below 300 g, except for "tap" stimuli as described below. Also, no units responded to thermal stimulation below 43°C, and responses to TCES did not occur below 5 mA. Excitatory responses predominated with each type of stimulus, although occasional units with pure OFF or inhibitory responses were encountered.

TABLE 1. *Number of units analyzed in anterior cingulate cortex*

	Noxious TCES	Noxious Mechanical	Noxious Thermal
Total units tested	542	221	47
Responsive units	150	93	9
Excitatory responses	130	85	7
Inhibitory responses	20	8	2

TCES, transcutaneous electrical stimulation.

Of the 47 units that were tested for responses to noxious heat stimuli, 36 units also had responses to noxious mechanical stimuli. Of these units 33 were excited, and the activity of three was reduced by noxious mechanical stimuli. Seven of the units with excitatory responses also responded to noxious heat stimuli, whereas two of the three units with inhibitory responses also responded to these thermal stimuli. Thus 25% of units with responses to noxious mechanical stimuli were polymodal units. No units responded exclusively to noxious heat stimuli.

The only innocuous stimulus that activated cingulate neurons was a tap. These stimuli were produced by lightly tapping the ear with a probe. The durations of such stimuli were ~100 ms with pressures of <150 g. Pinching stimuli with a similar intensity that lasted 2–10 s failed to evoke responses. In five rabbits, 14 units with responses to noxious TCES and pressure were tested with tap stimuli. Of these 14 units 11 responded to tap.

Figure 1 shows an example of the responses of a polymodal unit in anterior cingulate cortex. This unit did not respond to light mechanical stimulation nor to heat stimuli below 46°C. It did have an excitatory response to noxious TCES as shown in the raster display. This unit also responded to sharp pinch, i.e., with a serrated forceps, and tap. As also shown in this figure (⌓ under *C* TAP), an 8-s pinch with a smooth forceps at a pressure of ~150 g did not evoke a response. Finally, this unit responded to thermal stimuli ≥46°C.

Variability of response amplitudes to multiple presentations of noxious stimuli was evident in the unit for which responses are presented in Fig. 1. Response variability was common for nociceptive units in cingulate cortex and made it difficult to uncover responses that were graded with the intensity of mechanical stimuli to the contralateral ear. Fig-

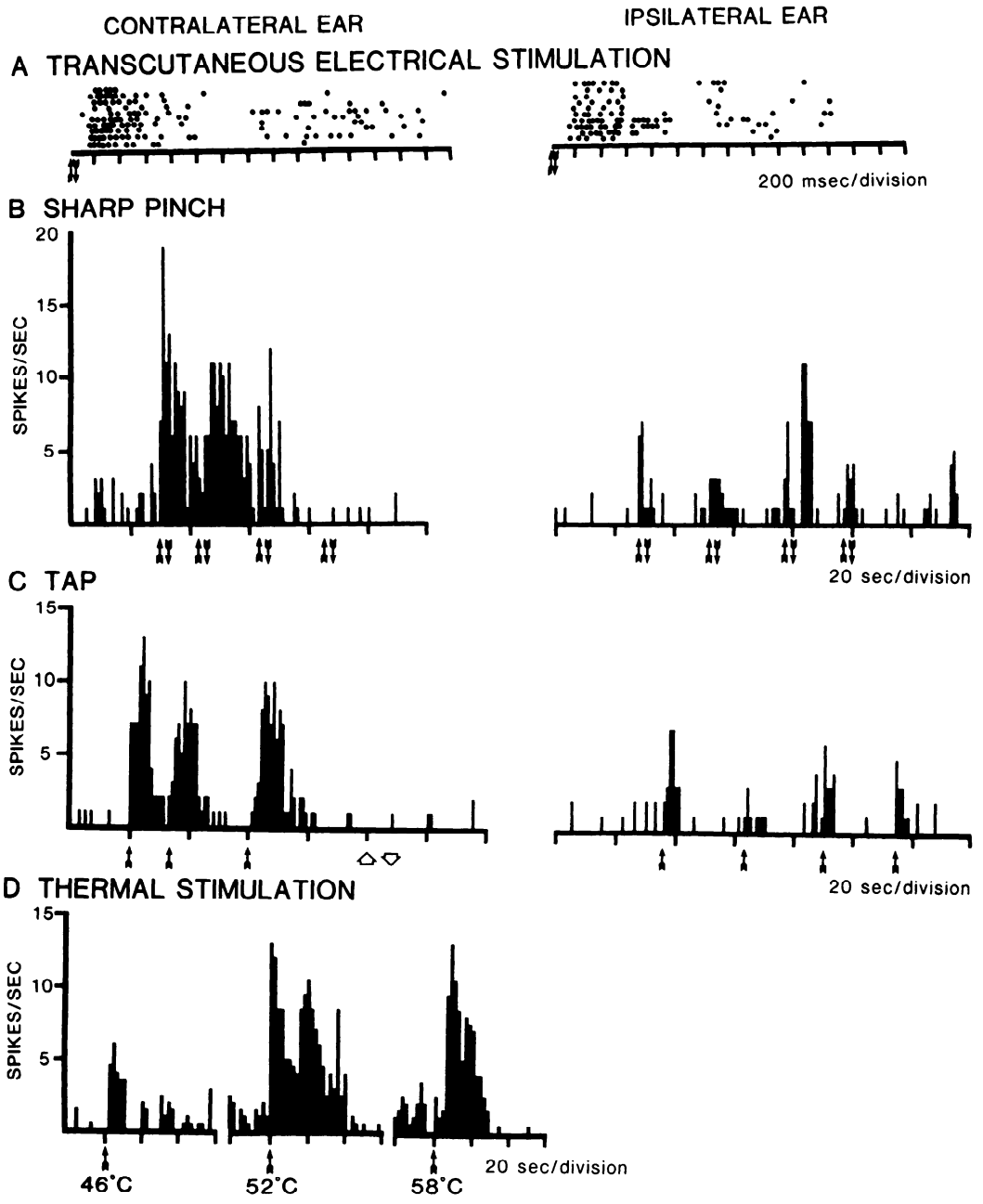

FIG. 1. Responses of a unit in area 24b to noxious and "tap" stimuli to the contralateral and ipsilateral ears. A raster for 10 sweeps after 9 mA transcutaneous electrical stimulation (TCES) is shown in which there is a prominent initial excitation, a longer latency period of inhibition, and then a weak excitatory response. Pinches with a serrated forceps evoked responses in both ears, although those to the contralateral ear were more robust. Tap stimulation evoked responses, whereas a pinch with a smooth forceps failed to evoke a response (⌓). Finally, heat stimuli over 45°C evoked responses in this unit including those shown for 46, 52, and 58°C.

ure 2 shows the responses for five units for which there were five to seven levels of mechanical stimulation. A one-way ANOVA was significant ($F = 3.48$, $P = 0.017$), indicating that these cells had a significant response to noxious stimulation. To determine whether significant changes in the responses were produced by the different amplitude stimuli, the Newman-Keuls test was used. No significant changes were found for stimulation with the 100- to 750-g stimuli ($P \leq 0.05$), however, 1000-g stimuli elicited responses that were significantly greater than those of ≤750 g. The magnitude of the responses declined with more intense stimuli, but this decline was not significantly different from that evoked with the 1,000-g stimuli.

There were 70 units for which 10–30 consecutive 10-s sweeps were available for statistical analysis of excitatory responses to noxious TCES of the contralateral ear. The onset and offset of a response was determined from a 10-bin moving average of responses in comparison with the baseline mean. A response onset was defined as the initial point at which the differences between these averages were significant at the 95% confidence level, whereas the offset of responses was defined as that point at which these averages

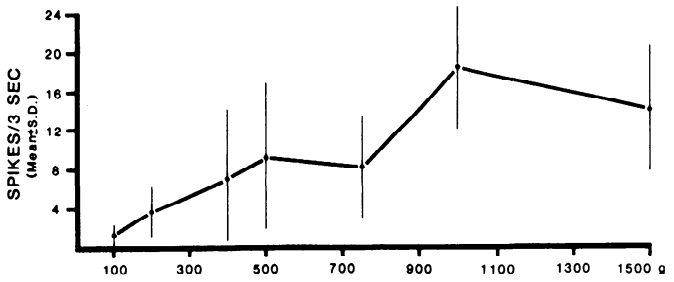

FIG. 2. Responses to a graded series of mechanical stimuli for 5 units. The response was calculated by subtracting average baseline activity previous to the stimulus from 3 s of the response. The mean value for each stimulus amplitude was calculated for each unit and the mean ± SD calculated for all units.

were no longer significant. The average rate of spontaneous activity throughout these recording sessions was 3.1 ± 0.39 (SE) spikes/s and the average response to TCES was 10.1 ± 0.81 spikes/s. The latter average represents the mean sustained response to each stimulus presentation. The maximum response was defined as the discharge frequency during the most vigorous response of an individual unit. The maximum response for all units was 20.5 ± 2.5 spikes/s. The average peak response to noxious TCES for these units was 16.1 ± 1.2 spikes/s per 50-ms bin averaged over 10 sweeps. Response onset for these 74 units occurred 166 ± 11.3 ms after TCES with a range of 50–600 ms and had a duration of 519 ± 52.1 ms with a range of 21–2,040 ms.

The histogram of the latency of responses to noxious TCES in Fig. 3 shows that this is a unimodal distribution. Figure 3 also has a histogram of the duration of these responses, and this distribution is unimodal and positively skewed. To assess the relationships among the response properties of these units, correlation coefficients were calculated. Examples of some of the correlations that were tested included response latency and magnitude of the sustained discharge ($r = 0.17$), peak response amplitude and response duration ($r = 0.22$), and the duration of the response and its standard deviation as a measure of response variability ($r = 0.29$). Only the last of these correlations was significant, and all other correlations that were considered had coefficients close to zero.

The raster of unit responses to contralateral TCES presented in Figure 1 shows that, after a 400-ms excitatory response, there was a period of ~590 ms when this unit did not respond. Forty-six units had an adequate spontaneous rate, i.e., >3 spikes/s, so that periods of inhibition could be detected qualitatively and quantitatively. Of these units, 10 had statistically significant OFF periods with a latency of 706 ± 195 ms and a duration of 885 ± 242 ms. A secondary period of excitation was present in six units with a 1,711 ± 313-ms latency and a 616 ± 99-ms duration.

Of the 150 units that responded to noxious TCES, 20 had reduced spike discharges. Most of these OFF responses were of short duration lasting 600-1,000 ms as was the case for 17 units. An example of the brief period of inhibition is shown in Figure 4*A*. The response of this unit to sharp pinch occurred during the stimulus presentation and there were no responses to noxious heat stimuli. The remaining three units that were inhibited by noxious TCES were off for periods lasting as long as 30 s, as shown in Figure 4*B*. In this latter unit a sharp pinch and heat ramp from 28 to 55°C shut activity off for over 20 s.

Organization of receptive fields

Sensitivity to noxious mechanical stimuli was tested over the dorsal body surface of 10 rabbits. All of the 15 units tested had broad receptive fields. For 12 of these units, excitatory responses were evoked by noxious mechanical stimuli applied anywhere on the dorsal surface as shown for one unit in Figure 5. In three instances, units responded only to noxious mechanical stimuli applied to the ears, forepaws, and back of the rabbit. An example of one of these units is shown in Figure 6.

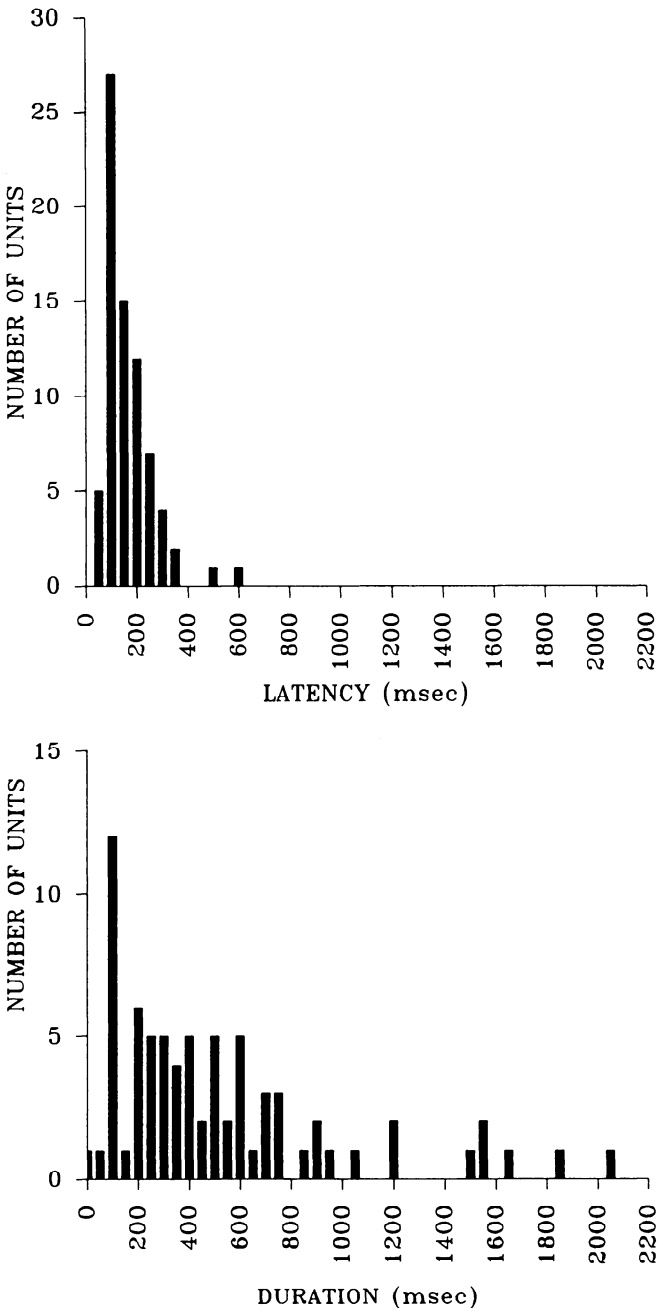

FIG. 3. Histograms of the latency and duration of initial excitatory responses to noxious TCES of the contralateral ear for 74 units.

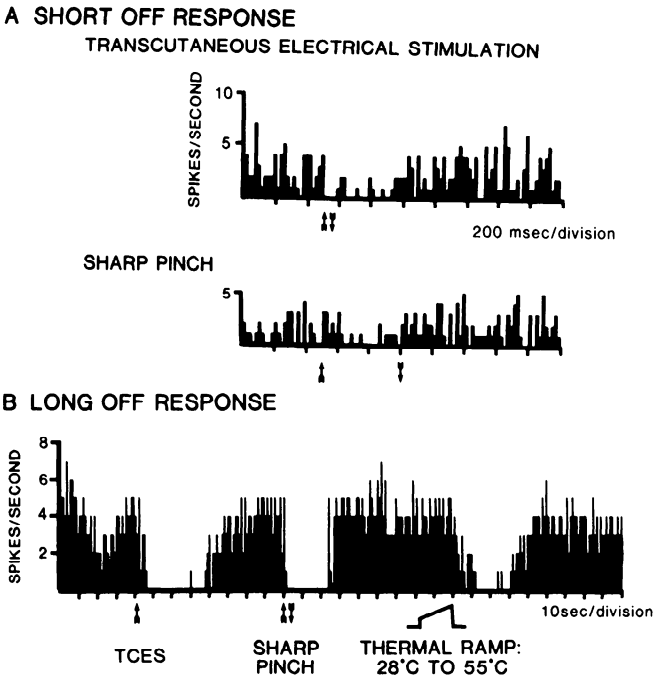

FIG. 4. Most OFF responses to noxious stimuli were of short duration like that for the first of these 2 units. *A*: unit was inhibited for >400 ms after a 50-ms, 10-mA TCES to the contralateral ear. A 250-ms pinch with serrated forceps also produced a weak but statistically significant decrease in activity during the stimulus. Heat stimulation had almost no effect on the spontaneous activity of this unit. *B*: a few units had a prolonged OFF response to noxious stimuli as shown for the second unit. This unit was OFF for 35 s after an 8-mA TCES and for >20 s after the sharp pinch and heat ramp stimuli.

In the examples of unit responses in Figures 1, 5, and 6 there is an indication that responses to contralateral stimuli might be greater than those to ipsilateral stimuli. There were an adequate number of trials for statistical analysis of bilateral TCES to the ears for 33 units with excitatory responses. In 15 units there were no significant differences between responses to contralateral and ipsilateral TCES (t test, $P > 0.05$); thus, there was no laterality in the responses. For 10 units the responses to contralateral stimuli were significantly greater than those to ipsilateral responses, and for 8 units the responses to ipsilateral stimuli were greater than those for contralateral stimuli. The locations of these units in superficial or deep layers were histologically confirmed for 26 units and estimated for the other 7 with depth and midline measurements. This analysis showed that nine units in superficial layers had no laterality, whereas seven units had a contralateral, and one had an ipsilateral bias in their responses. In contrast, in deep layers six units had no laterality, three had a contralateral, and seven had an ipsilateral bias.

Location of units in area 24b

There were 737 units isolated throughout the dorsal and medial surfaces of rabbit cortex. Of these units 186 were localized histologically as discussed below, and the remainder were located with stereotaxic coordinates in relation to identified electrode tracks. Figure 7 shows the distribution of nonresponsive units and those that responded to noxious TCES and/or noxious mechanical stimuli on the dorsal and medial surface of the rabbit brain with reference to bregma, the midline, and the rostrum of the corpus callosum. Responsive units were congregated in the rostral and dorsal part of area 24b with some overlap into area 8. These latter units are discussed briefly below.

The caudal and ventral borders of a "nociceptive region" in anterior cingulate cortex are emphasized in Figure 7 (*****). There were 110 responsive and 125 nonresponsive units in cingulate cortex between the asterisks and the border of areas 24b and 8. In cingulate cortex ventral and caudal to these asterisks there were 47 responsive units and 269 nonresponsive units. The proportion of responsive to nonresponsive units was 88% in the nociceptive region of cingulate cortex, whereas this proportion was 17.5% for cingulate cortex that was ventral and caudal to the nociceptive region. Therefore, the topographical distribution of nociceptive units in cingulate cortex was not a simple function of the sampling procedure.

Table 2 shows the laminar distribution for 186 units that could be histologically verified with either a lesion at the end of an electrode penetration or with an electrode track. The highest percentage of responsive units were in layer III where 43% of the isolated units had responses. In layer II, 28% responded to noxious stimuli, whereas in layer V only 17% responded. None of the four units in layer VI responded to noxious stimuli. The units in layer V were usually superficial near layer III. χ^2 analysis of the distribution of units in layers II-V showed that this distribution was significantly different from homogeneity ($\chi^2 = 8.7$, $P < 0.02$). Finally, the laminar distribution of excitatory and

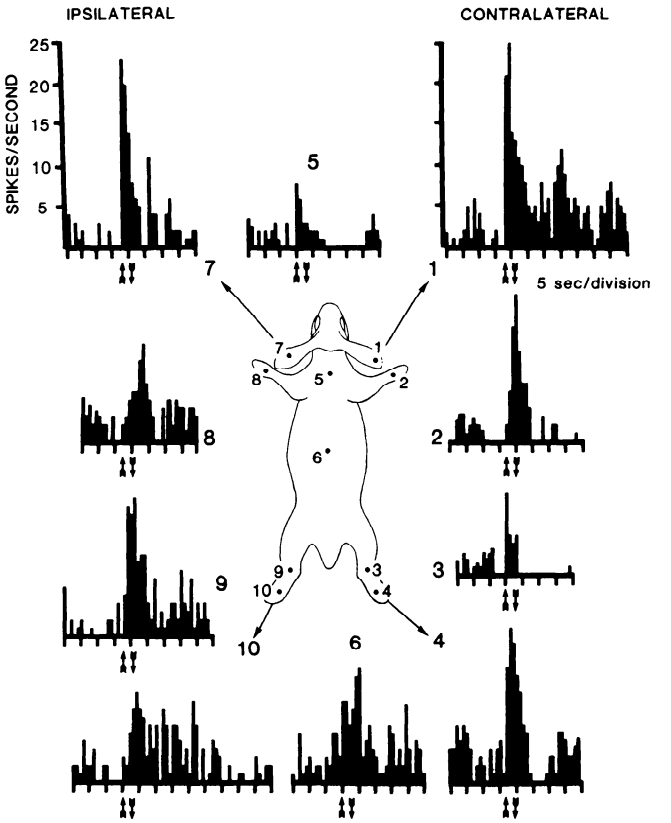

FIG. 5. Most units in anterior cingulate cortex responded to noxious stimuli on the entire body surface of the rabbit as was true for this one. Noxious mechanical stimuli were delivered with serrated forceps for 3 s on all parts of the dorsal surface of this animal, and all stimuli excited this unit.

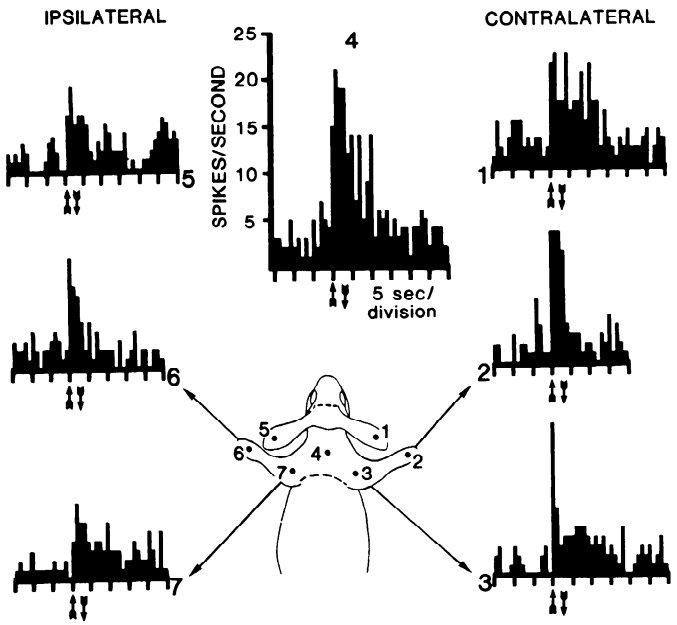

FIG. 6. The responses of this unit reflected those for a limited number of anterior cingulate units that were excited by stimuli to part of the rostral body surface. This unit responded maximally to 3-s pinches with serrated forceps in the region between the dashed lines. No responses could be produced by stimulations of the dorsal surface of this rabbit caudal to the second dashed line.

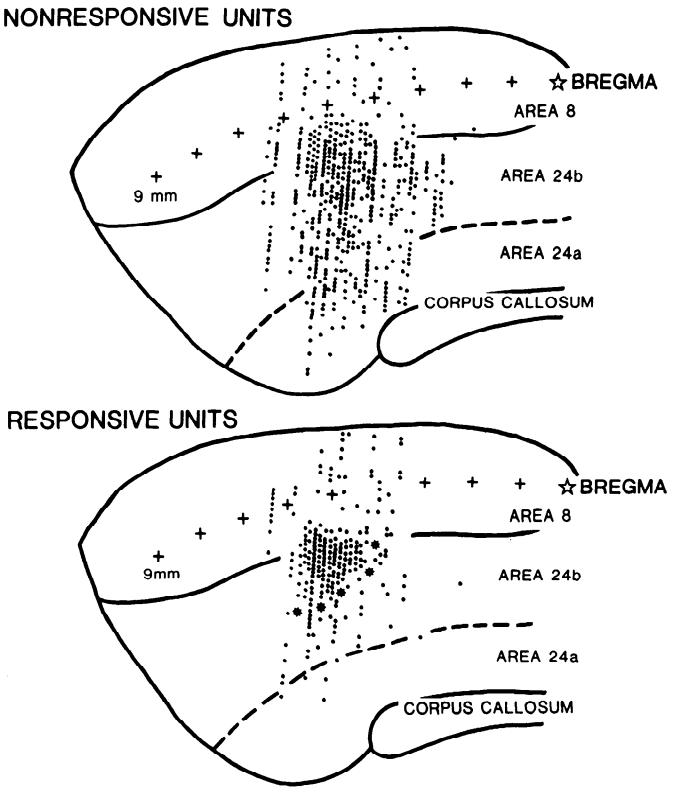

FIG. 7. These maps are flattened representations of the rostral cerebral cortex with areas 8, 24b, and 24a delineated. *Top*: each dot identifies a unit that was isolated and did not respond to noxious TCES or noxious mechanical stimuli. *Bottom*: each dot represents a unit that responded to these stimuli on the contralateral ear. Bregma is represented with a star, and each millimeter anterior to bregma is represented at the midline with a + for a distance of 9 mm. The genu of the corpus callosum served as an important reference for the reconstruction of the positions of each of these units. Location of responsive units was contained within the area from which nonresponsive units were isolated. The area with the highest density of nociceptive neurons was in a dorsal and rostral part of area 24b that overlapped with area 8. *****, a critical point in cingulate cortex ventral and caudal to which many units were isolated but few were responsive.

inhibitory units was about the same. In layer II, 77% of the units were excited, whereas 89% of the units in layers III and V were excited.

Nociceptive units in area 8

Vertical electrode penetrations of cingulate cortex necessarily passed through area 8. Because the locations of all isolated units were recorded as were the presence of responses to noxious TCES, these data are included in Figure 7. A thorough comparison of these responses cannot be made with those of nociceptive neurons in cingulate cortex because a complete testing procedure was not conducted on the area 8 units. There were eight units in area 8, however, for which response latency to noxious TCES was assessed. Noxious, TCES-evoked excitatory discharges in these units had a latency of 144 ± 22.6 ms, and each of these units responded to noxious mechanical stimulation.

The topography of responsive units shown in Figure 7 suggests that there was a high proportion of responsive units in area 8. Histological assessment of the location of these units indicated that of the responsive units 21 were in superficial and four in deep layers, whereas 21 nonresponsive units were in superficial and 10 in deep layers. The apparently high density of nociceptive units in area 8 was likely influenced by the sampling procedure for cingulate units. Thus the electrode tracks almost always passed through superficial layers of area 8 in the course to cingulate cortex, the electrodes passed more frequently through the deep layers of area 24 than those of area 8, and nonresponsive units were not thoroughly studied in area 8.

Experimental studies

There are two likely sources of the nociceptor-generated signals in anterior cingulate cortex. Although direct projections from somatosensory to cingulate cortex have not been described in the rat or rabbit, it is possible that indirect connections from somatosensory cortex could course through medial granular cortex, i.e., those of area 8 to area 24 (Reep et al. 1990; Vogt et al. 1986). In addition, as discussed in the introduction, nociceptor-generated activity could pass through the medial and/or intralaminar thalamic nuclei. Thus two experimental preparations were employed to assess these possibilities: cortical knifecut lesions or lidocaine injections into the thalamus.

TABLE 2. *Laminar distribution of 186 histologically verified units*

	Responsive Units	Excitatory Responses	Inhibitory Responses	No Responses	Total Units Isolated	Responsive Units, %
II	13	10	3	34	47	28
III	35	31	4	47	82	43
V	9	8	1	44	53	17
VI	0	0	0	4	4	0
Total					186	

Knifecut lesions were made lateral and posterior to area 24b to remove inputs to anterior cingulate cortex from ipsilateral neocortical areas and from area 29d without also interrupting thalamic afferents to area 24 that course rostrally through the cingulum bundle. An example of one of these cases is shown in Figure 8. In the electrode track illustrated there were two units that responded to noxious TCES and mechanical stimuli. Responses for the ventral of the two units are shown in this figure and responses occurred to both ipsilateral and contralateral TCES to the ear. In this instance the response to the ipsilateral stimulus was slightly greater than that to the contralateral stimulus. There were 61 units isolated in rostral area 24 of five rabbits after the knifecut lesions, and 9 of these units had excitatory responses to noxious stimuli. Before the lesions, 50 units were tested of which 11 had excitatory responses and 3 had inhibitory responses. The differences in the percentage of driven neurons in pre- vs. postlesion animals was not statistically significant ($\chi^2 = 2.94$, $P = 0.1$). There were no apparent change in the characteristics of the responses to noxious stimulation after the lesion. The latency of the response was 157 ± 12 ms and was not significantly different from the prelesion latency. Of the nine responsive units, six were tested with noxious mechanical, and two were tested with noxious heat stimulation. All six units responded to the mechanical stimulation, whereas neither of the units tested responded to the noxious heat stimuli.

The medial thalamus was explored with a recording electrode to identify a region in which responses to noxious stimuli could be evoked. A unit was then isolated in cingulate cortex that also responded to noxious stimuli. Once the cortical responses were characterized, 1–2 μl lidocaine injections were made into the thalamus with a cannula that was attached to the thalamic recording electrode. For all 12 cingulate units tested in six animals the cortical responses were almost completely abolished, and there were no effects on cortical responses after five injections of 0.9% NaCl. Figure 9 shows electrode track reconstructions for five rabbits and two unit responses in cingulate cortex before and after lidocaine injections; tracks from a sixth case were not recovered. In the first case shown in Figure 9 the recording electrode in the thalamus was in the submedial nucleus, and the injection cannula was in the ventral nucleus. The unit response in area 24b to noxious TCES of the contralateral ear was virtually abolished with lidocaine 350 s after two 1-μl injections. This response essentially recovered 1,450 s after the injections were terminated. In the second example shown in Figure 9, the thalamic recording electrode was in the parafascicular nucleus, and the injection cannula was dorsal to this nucleus. The unit response in cingulate cortex was abolished within 220 s of a single 1-μl injection of lidocaine. The response to noxious TCES partially recovered 1,360 s after the lidocaine injection; however, it was not equivalent to preinjection levels.

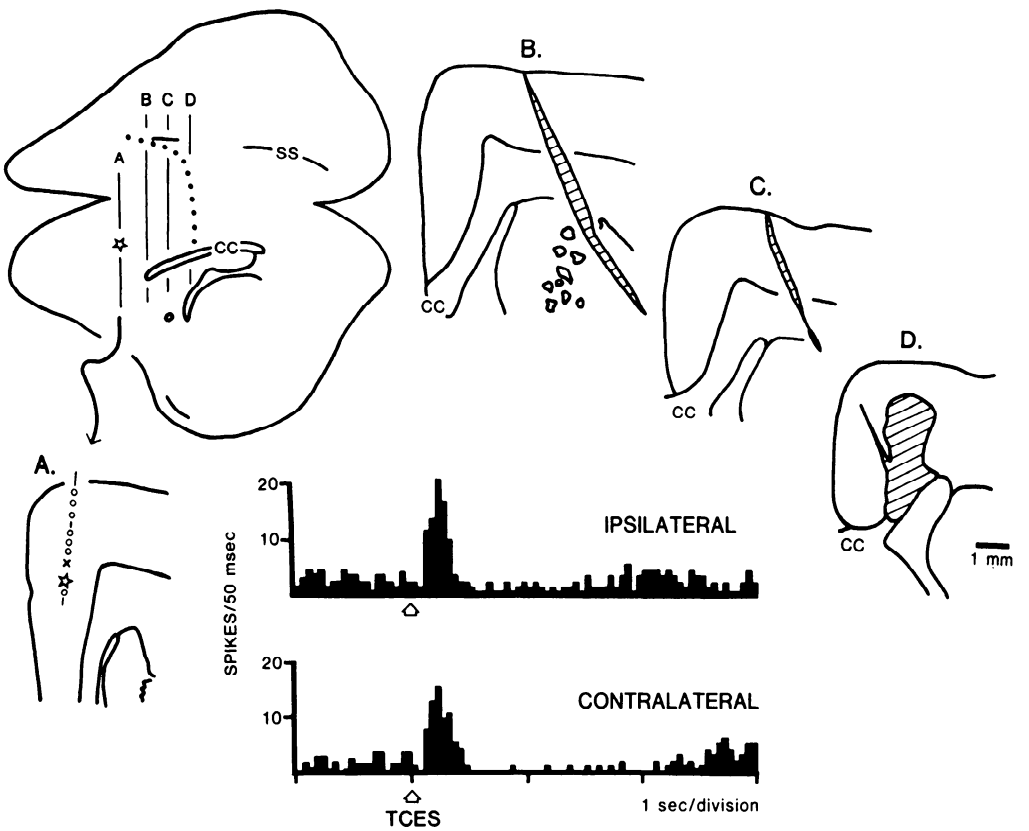

FIG. 8. Example of a case in which a cortical knifecut lesion was placed followed by an analysis of responses to noxious stimuli. On the surface reconstruction of this brain a dotted line represents the extent of the knifecut. CC, corpus callosum; SS, splenial sulcus. *A–D*: transverse sections with the star in *A* representing the location of the unit that responded to 8 mA TCES of the ipsilateral and contralateral ears. *B–D*: hatched areas represent the knifecut lesion lateral to the recording site. Two units in this electrode penetration responded to noxious TCES.

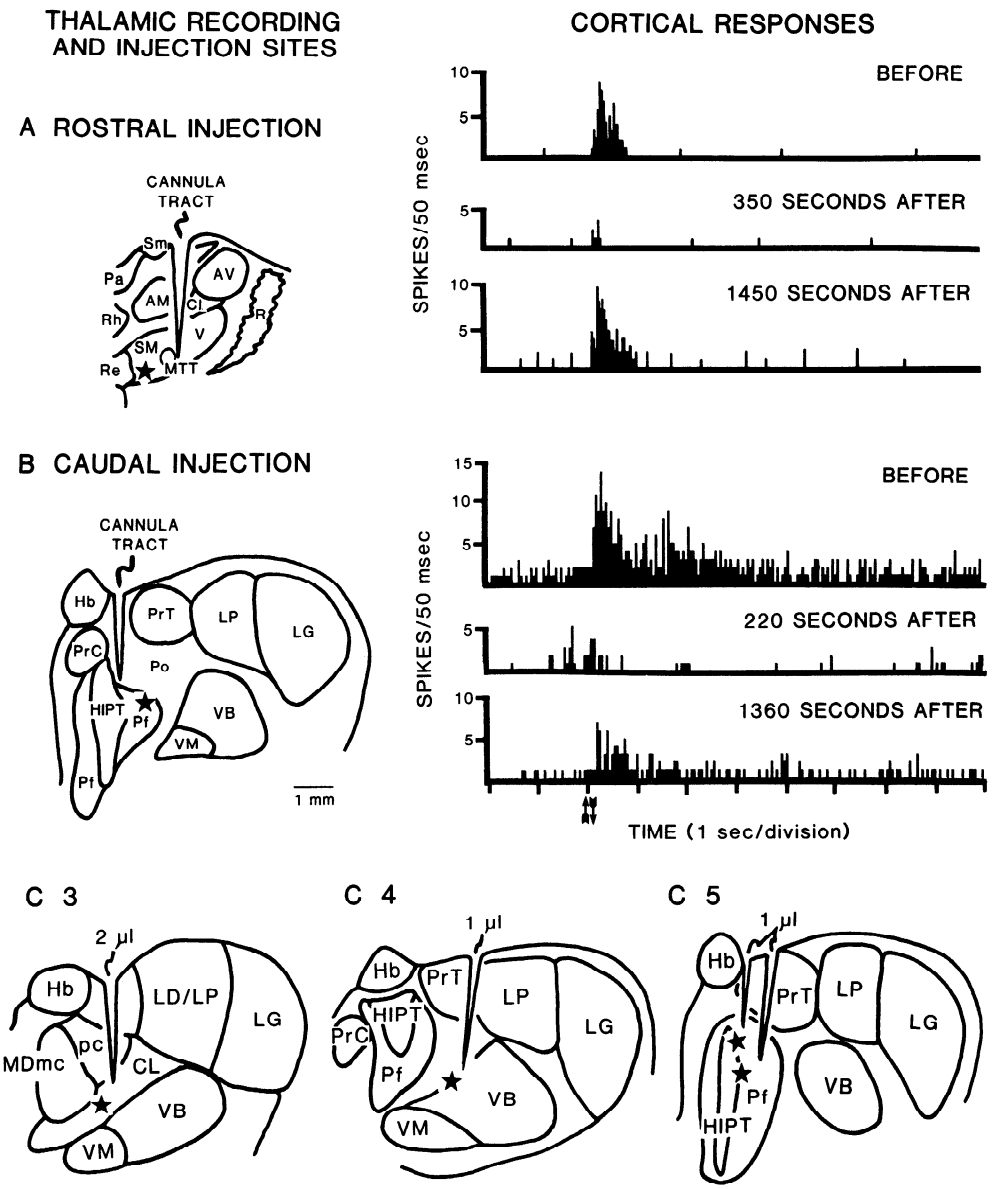

FIG. 9. Reconstructions from animals in which a cannula with a recording electrode attached was placed into medial parts of the thalamus. The injection cannulae were localized with noxious, TCES-evoked activity in the thalamus (★). In the first electrode placement, the nociceptive thalamic unit was in the submedial nucleus (SM), and the injection cannula was lateral to this site in the ventral nucleus. The cortical responses to TCES were virtually abolished with 2 µl of lidocaine and returned completely within 1,450 s. In a second electrode placement, the responsive thalamic unit was in the parafascicular nucleus (Pf), and the injection cannula was dorsal and medial to this site. Only 1 µl of lidocaine was required to virtually abolish the cortical responses to TCES, and after 1,360 s the responses had still not recovered to preinjection levels. *C*: reconstruction of 4 other injection sites from 3 cases and the amount of lidocaine that was required to block nociceptive neuronal activity in anterior cingulate cortex. AM, anteromedial; AV, anteroventral; Cl, centrolateral; HIPT, habenulopeduncular tract; LD, laterodorsal; LP, lateroposterior; LG, lateral geniculate; MDmc and pc, magnocellular and parvicellular mediodorsal; Pa, paraventricular; PO, posterior; PC, precommissural; PrT, pretectal; V, ventral; VB, ventrobasal; VM, ventromedial; R, reticular; Re, reuniens; Rh, rhomboid; Sm, stria medullaris.

Figure 9 also shows the locations and effective doses of lidocaine for four other injection sites. In a third case (Fig. 9*C3*), the responsive thalamic unit was in the centrolateral nucleus. To block the activity of four cingulate units from this site, injections of 2 µl of lidocaine were required. In a fourth case (Fig. 9*C4*), the responsive thalamic unit was on the border of the ventrobasal and posterior nuclei and only 1 µl of lidocaine was necessary to block a cingulate unit's activity. In a fifth case (Fig. 9*C5*), a medially placed cannula was in the parafascicular nucleus and the activity of three cingulate units was blocked with 1-µl injections, whereas a laterally placed cannula in the same nucleus also required 1 µl of lidocaine to block the activity in two cingulate units. Thus 2-µl injections of lidocaine were required into rostral levels of the thalamus to block nociceptive unit activity in cingulate cortex, whereas only 1-µl injections

were required when the cannula was in or just rostral to the parafascicular nucleus and these latter injections produced blocks that lasted for longer periods of time.

DISCUSSION

Neurons in anterior cingulate cortex responded to noxious mechanical and heat stimuli. There was generally little somatotopic organization, although quantitative analysis showed that 55% of the units had preferential responses for either the contralateral or ipsilateral ear. Of the units that responded to noxious mechanical stimuli, 25% were polymodal with responses to noxious heat stimuli. Nociceptive units were aggregated in the superficial layers of dorsal cingulate area 24b and overlapped with the medial part of area 8, and responses were very sensitive to thalamic lidocaine injections.

The response properties of anterior cingulate cortical neurons are similar to those reported for neurons in the parafascicular, centrolateral, and submedial nuclei of the thalamus. Neurons in these nuclei respond primarily to noxious stimuli, have little or no somatotopic organization, and have a high proportion of neurons that respond to innocuous "tap" stimuli (Dong et al. 1978; Miletic and Coffield 1989; Peschanski et al. 1981). In addition, Dong et al. (1978) showed weak coding for mechanical stimuli in the thalamus of the anesthetized cat and Bushnell and Duncan (1989) observed coding for heat stimuli between 46 and 49°C in the alert monkey. Although the present study does not provide evidence for intensity coding, it is possible that cingulate neurons have a narrow intensity coding range for noxious heat stimuli.

Dong et al. (1978) showed that electrical stimulation of peripheral nerves evoked two periods of excitation with 70- and 300-ms latencies in cat medial thalamic neurons. In addition, they demonstrated that these periods of excitation were driven independently by Aδ and C fibers, respectively. Furthermore, Craig and Kniffki (1985) reported that lamina I spinothalamic projection neurons had central conduction latencies of 63 ms for units driven by Aδ fibers, whereas latencies were 135 ms for those driven by C fibers. These long-latency responses are compatible with the long-latency excitatory responses evoked in nociceptive cingulate neurons in the rabbit of 166 ± 11.3 ms. A small number of cingulate neurons had a second period of excitation with a latency of 1,711 ± 313 ms. This second period of excitation is likely influenced by multisynaptic excitatory and inhibitory circuits in cingulate cortex and/or by connections between cingulate and other cortical regions.

It is possible that nociceptive responses in cingulate cortex are transmitted through the medial and intralaminar nuclei of the thalamus for the following reasons. First, neurons in these nuclei respond to noxious stimuli in a manner that is similar to neurons in anterior cingulate cortex as discussed above. Second, thalamic nuclei that project to cingulate area 24 include the parafascicular, centrolateral, and submedial nuclei in addition to other nuclei that receive spinothalamic afferents such as the reuniens and ventromedial nuclei (Baleydier and Mauguiere 1980; Musil and Olson 1988; Robertson and Kaitz 1981; Vogt et al. 1979, 1987, 1992). Third, lidocaine injections into the medial thalamus virtually abolished nociceptive responses in all units tested in anterior cingulate cortex.

An alternative source for nociceptive responses in cingulate cortex could be from other cortical regions. Surgical lesions that removed multisynaptic input to area 24 from somatosensory, insula, parietal, and posterior cingulate cortices failed to abolish responses to noxious stimuli in the present study. Nociceptive inputs, however, could arise from areas 25 or 11 (area VLO in the rat; Krettek and Price 1977), and the projections of these areas were not involved in the present series of lesions in part to avoid involvement of thalamic afferents that pass through the cingulum bundle. Areas 25 and 11 have weak projections to dorsal parts of anterior cingulate cortex in the rat and monkey (Vogt and Miller 1983; Vogt and Pandya 1987), and each receives input from the parabrachial nucleus in the rat (Saper and Loewy 1980). Because the parabrachial nucleus itself receives direct inputs from lamina I nociceptive neurons in the spinal cord (Hyden et al. 1986), areas 25 and 11 could transmit nociceptive information to dorsal cingulate cortex. Furthermore, VLO in cat receives submedial input (Craig et al. 1982) and so VLO could serve as a source of nociceptive information for cingulate cortex. One note of caution for this corticocortical hypothesis is the fact that areas 25 and 11 have not yet been shown in positron emission tomography studies to have elevated blood flow during noxious stimulation when cingulate cortex is active (Jones et al. 1991; Talbot et al. 1991). Thus these projections are not necessarily relevant to cingulate neuronal responses to noxious stimuli.

Histological localization of the nociceptive units in anterior cingulate cortex showed that most were in area 24b. The greatest number of units were in layer III where 43% of the isolated units responded to noxious stimuli. Only 28% of isolated units in layer II and 17% in layer V responded to noxious stimuli. In addition, many of the layer V neurons were very close to the border with layer III. Although the mediodorsal nucleus projects primarily to the inner part of layer III in area 24 (Vogt et al. 1981), it appears that projections of the centrolateral (Cunningham and Levay 1986), ventromedial, and submedial (Herkenham 1979) nuclei are to layer I of area 24. Therefore, the most direct means of activating neurons in layer III by a layer I projection is via terminations on the apical tuft dendrites of layer III pyramidal neurons. Also, because nociceptor stimulation evoked an initial period of inhibition in a small percentage of units, it may be that the dendrites of interneurons also receive a direct input from medial thalamic nuclei and contribute to subsequent inhibitory responses. It is possible, therefore, that the interruption of the excitation to form two bursts observed for a number of neurons excited by TCES is the result of direct excitation of cortical inhibitory interneurons.

Although receptive fields for cingulate cortical neurons were broad according to qualitative assessment, statistical analysis of numerous TCES to the ear showed that 18 of 33 units so tested had a significant lateral preference. Ten units with a contralateral preference were primarily in layer III, whereas 8 units with an ipsilateral preference were in layer

V. Similar preferences may also occur in medial and intralaminar thalamic neurons (e.g., Kayser and Guilbaud 1984). If there is a bias in some thalamic responses for ipsilateral or contralateral stimuli, then cortical responses might be accounted for on the basis of the relationships between cortical neuron dendrites and thalamic afferents. It is also possible that such a circuitry is modulated by connections from the contralateral hemisphere. Contralateral callosal connections in area 24b are most dense to layers Ib-III (Vogt et al. 1981; Vogt and Gorman 1982), whereas callosal inputs are minor in layer V.

Behavioral and neurosurgical observations suggest that cingulate cortex is involved in avoidance conditioning (Gabriel et al. 1991a; Lubar 1964; Lubar and Perachio 1965; Peretz 1960; Thomas and Slotnick 1963) and affective responses to noxious stimuli (Amromin et al. 1975; Ballantine et al. 1987; Foltz and White 1962; Turnbull 1972; White and Sweet 1969), respectively. In spite of the fact that halothane depresses somatosensory responses (e.g., Dykes and Lamour 1988), the present study shows that neurons in a dorsal part of area 24b respond directly to noxious mechanical and heat stimuli and that these responses have little stimulus localization information. In addition to their possible role in the affective assessment of noxious stimuli, cingulate cortex could contribute to various autonomic responses associated with noxious stimuli. It has long been known that electrical stimulation of anterior cingulate cortex in experimental animals and human cases evokes many visceral and autonomic responses such as mydriasis, increases and decreases in heart rate and blood pressure, vocalization and inhibition of respiration (reviews: Devinsky and Luciano 1993; Neafsey 1990). Furthermore, neurons in the dorsal and anterior part of area 24 undergo training-induced changes in activity during aversive classical conditioning (Gibbs and Powell 1991). In this latter study changes in neuronal activity appeared to reflect the differential contingencies of the positive and negative conditional stimuli and there were strong correlations between tone-evoked multiunit activity and heart rate conditioned responses. Thus, nociceptive responses of cingulate neurons may contribute to affective responses to noxious stimuli and/or homeostatic processes.

There are numerous pathways by which anterior cingulate cortex can influence somatic and autonomic motor activity in response to nociceptive activity. These projections include those of area 24 to the caudate and pontine nuclei (Gerfen 1989; Royce 1982; Wiesendanger and Wiesendanger 1982; Yeterian and Van Hoesen 1978), the periaqueductal gray (Morrell et al. 1981), and the parafascicular nucleus of the thalamus (Royce 1983). Neurons in area 25 of cingulate cortex also project to the nucleus of the solitary tract, dorsal motor nucleus of the vagus nerve, and the intermediolateral cell column in the spinal cord (Hurley et al. 1991).

In light of the connections and neuronal response properties of cingulate cortex, it is possible that anterior cingulate cortex is directly involved in affective and/or autonomic responses to noxious stimuli. These functions likely include a contribution to learning processes that require the prediction of noxious stimuli via its sensory connections and behavioral avoidance of painful stimuli by its projections into somatic and autonomic motor systems.

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