VAGAL INPUT TO LATERAL AREA 3a IN CAT CORTEX

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ABSTRACT

Penfield's sensory homunculus included visceral organs at its lateral extreme, and vagal input was recently identified lateral to the intraoral representation in primary somatosensory cortex (S1) of rats. We tested whether vagal input is similarly located in cats, where area 3b (equivalent to S1) is clearly distinguishable from adjacent regions. Field potentials were recorded from the intact dura over the left hemisphere using electrical stimulation of the left or right cervical vagus nerve in seven cats. A surface positive-negative potential was evoked from either side in the lateral part of the sigmoid gyrus. Finer mapping made at the pial surface with a microelectrode identified a focal site anteromedial to the anterior tip of the coronal sulcus. Depth recordings demonstrated polarity reversals and multi-unit vagal responses, indicating that the potentials were generated by an afferent activation focus in the middle layers of the cortex. The S1 mechanoreceptive representation was localized by mapping multi-unit somatosensory receptive fields in the middle cortical layers near the coronal sulcus. The vagal evoked potential site was distinctly anterior to the intraoral S1 representation and adjacent to the masseteric nerve evoked potential focus. Lesions made at the focal site revealed that this site is cytoarchitectonically located in area 3a, not area 3b. Thus, vagal input to the sensorimotor cortex in cats resembles deep rather than cutaneous somatic input, similar to the localization of nociceptive-specific input to area 3a in monkeys. The possibilities are considered that this vagal input is involved in motor control and in the sensory experience of visceral afferent activity.
INTRODUCTION

Application of non-invasive functional imaging methods to the human brain have demonstrated the involvement of numerous cortical regions in visceral sensation. Particularly, they have confirmed Penfield's sensory homunculus, which included an abdominal representation in its most lateral part (Penfield and Rasmussen 1950). Functional magnetic resonance imaging (Aziz et al 2000b; Binkofski et al 1998), magnetoencephalography (Furlong et al 1998; Hecht et al 1999), and positron emission tomography (Aziz et al 1997; Ladabaum et al 2001) have demonstrated activation in the corresponding region of the human cerebral cortex in response to esophageal or stomach stimulation. Thus, there seems to be a region in the neighborhood of the primary somatosensory cortex (S1) which receives sensory input from abdominal organs and might be involved in conscious sensations from these organs. However, this region has not been anatomically or functionally identified, and the afferent pathway responsible for this abdominal activation has not been determined. Furthermore, it contrasts with the standard view that visceral sensation involves primarily the insular cortex (Cechetto and Saper 1990).

Very recently, it was reported that the most lateral part of S1 in rats receives input from vagal afferents (Ito 2002). This provided the first anatomically identified demonstration of this putative visceral region in experimental animals. The results suggested that this region is continuous with the intraoral trigeminal representation in S1. However, these results did not provide a direct comparison with human cortex, because the organization of S1 cortex differs substantially between rat and human. While the sensorimotor cortex in rats seems to be a combined entity (or a mosaic) with respect to submodalities of somatic input (Gioanni 1987), the corresponding cortex in many mammals including humans has distinct cytoarchitectonic areas that receive input from different submodalities. In particular, area 3b receives cutaneous mechanoreceptive input and is considered to be S1 proper, and area 3a receives proprioceptive input and is considered an adjunct of motor cortex (Jones 1985; Kaas 1993; Weisendanger and Miles 1982). To address the issue of an S1 visceral region across species, it is crucial to clarify whether the vagal input
arrives in area 3a, area 3b or both. If vagal input is directed to area 3b, or S1, this would support the implication of Penfield’s homunculus that S1 is involved in the perception of visceral sensations. On the other hand, if the vagal input is received by area 3a, a fairly different scheme of the visceral sensory system would emerge, since area 3a is distinct from area 3b not only in the sensory submodalities represented but also in the cortical networks involved. In addition to proprioceptive input, area 3a receives nociceptive afferent input (Craig, 1995; Tommerdahl et al. 1996) that may be important for perception (Perl, 1984). Thus, the distinction of the area of sensorimotor cortex that receives vagal afferent input impacts the possible role of these regions in motor control and sensation.

With regard to this issue, cats have several advantages; they have distinctively different cytoarchitectonic areas (Avendano and Verdu 1992; Ghosh 1997; Hassler and Muhs-Clement 1964; Leclerc et al.1994) with well documented deep (area 3a) and cutaneous inputs (area 3b or S1 proper; Felleman et al.1983; Jones and Porter 1980). The S1 representation of the trigeminal intraoral region has been mapped (Iwata 1985; Taira 1987), which can guide localization of the vagal region (Ito 2002). Finally and most importantly, the vagal evoked potential in the region of sensorimotor cortex has been repeatedly examined in the cat. After Siegfried described the region for the first time (Siegfried 1961), others (Aubert 1970; Aubert and Legros 1971; Korn and Masson 1963; Massion et al. 1966) clearly differentiated the evoked potential in the lateral sigmoid gyrus, at the lateral extent of sensorimotor cortex, from the evoked potential focus in the orbital gyrus, which corresponds to the insular visceral region of humans, monkeys and rats. Unfortunately, these prior authors did not provide a cytoarchitectonic description, although they described the waveform, amplitude, latency or laterality in some detail. It was even left undetermined whether the evoked potential focus was in the somatosensory or motor portion of sensorimotor cortex.

In the present study we focused on identifying the cytoarchitectonic location of this vagal potential region in cats. The site was localized with microelectrode recordings from the pial surface and from the cortical depth, in order to demonstrate the generator site. Somatosensory
receptive fields were mapped in the nearby S1 to determine the relationship between the vagal input focus and S1. Finally, the cytoarchitecture of the site was determined. It is located in area 3a, not 3b. A preliminary report of this study was made (Ito and Craig 2002).

METHODS

Animal preparation

The experimental protocol was approved by the Institutional Animal Care and Use Committee, and all procedures complied with the guiding principles for the care and use approved by the American Physiological Society.

Seven cats of either sex weighing 2.4-4.5 kg were used; three were anesthetized with chloralose and four with Saffan (alphaxolone/alphadolone, Glaxo). Chloralose anesthesia (60 mg/kg iv) was preceded by ketamine (50 mg im) and supplemented with sodium pentobarbital iv as necessary. Animals that received Saffan anesthesia (12-18 mg/kg iv) were first tranquilized with acepromazine (0.3 mg/kg im); Saffan was continuously supplemented with infusion pump (8-12 mg/kg/hr). In all cats, a bolus injection of dexamethasone (10 mg iv) was given. The neck, head and face were shaved. A rectal thermistor was inserted, and an infrared lamp as well as a heated water pad was used to maintain core temperature at 37.5°C. Eye salve was applied, 0.5% bupivicaine was injected subcutaneously at incision sites, and a local anesthetic was sprayed in the ear canals. Blood pressure and heart rate were monitored with the catheter inserted in the right carotid artery (contralateral to the examined hemisphere). End-tidal CO2 was maintained at 3.5-4.5%. Depth of anesthesia was monitored with the width of the pupil and reflexive change in blood pressure and heart rate, and also with reflexes to pinch and corneal contact before introduction of paralytic agent.

After cannulation of the trachea and the carotid artery, the cervical vagus nerves were bilaterally isolated. Each nerve was attached with a cuff electrode consisting of two pairs of
platinum wires 30 or 50 mm apart; one pair was used for stimulation and the other for recording the compound action potential. The nerves were ligated distally to the electrode to avoid any vagal efferent-mediated responses confounding the cortical recording and to exclude possible contamination of sympathetic afferent activity (since the cervical vagus nerve may contain nerve fibers from the superior cervical ganglion).

Each animal's head was mounted in a stereotaxic frame so that the head was tilted with the left side up (only the left cerebral hemisphere was studied). The zygomatic arch, the postorbital processes, and the upper part of the mandible were removed. The eyeball was pulled ventral or, in one cat, removed. A craniotomy was made to expose the anterolateral cerebral cortex. Mineral oil or Tyrode’s solution were often applied to keep the exposed area from drying out.

**Vagal Stimulation/Recording**

The vagus nerves were electrically stimulated with either a single rectangular pulse (duration: 0.3 or 2.0 msec) or a train of pulses (three 0.3 msec pulses in 1 kHz). The stimulus strength was initially adjusted to be supramaximal for A-delta fiber activation in the compound action potential recorded from the vagus nerve; it produced C fiber activation as well (though the cortical response we recorded could not have resulted from C-fiber afferents based on the latency).

The cortical surface was photographed with a digital camera, and the recording sites were plotted on the printed photographs. The vagal evoked field potential was recorded with both low and high resolution methods. A low resolution map was made with tungsten or platinum macroelectrode to isolate the focal region and differentiate it from the orbital focus. The dura was left intact. The electrode was moved in two dimensions on a 1mm x 1mm grid with a manipulator which was independent of the stereotaxic frame and aligned nearly parallel to the cortical surface. For mapping, the intensity of vagal nerve stimulation was increased so that it was twice that needed to produce a maximal cortical evoked potential recorded at an arbitrary site near the coronal gyrus; it varied from 0.7 to 10.0 mA in the different cases.
In the four cats anesthetized with Saffan, high resolution microelectrode recordings were made after the rough mapping described above. The dura over the focal site was cut and reflected. A platinum-plated tungsten-in-glass microelectrode (tip size ~15 µm) was used to record the evoked potential from the pial surface. The cortex was often moving (due to respiration and arterial pulse pressure), so a slight pressure was usually imposed on the surface by the microelectrode, which produced a variable degree of dimpling. At the focal site, the laminar profile of the evoked potential was examined with the same microelectrode by advancing it and penetrating the cortex with steps of several hundred micrometers. The penetration angle was oblique to the cortical surface because of the curvature of the target cortex near the coronal sulcus.

The indifferent electrode was a silver wire wound in a disk shape or a stainless steel clip attached to nearby muscle. Signals were amplified with a bandpass of 1 Hz to 1 kHz for the macroelectrode and 10 Hz - 10 kHz for the microelectrode. Multi-unit spike activity was recorded with a bandpass of 300 Hz - 10 kHz. The signals were stored in a digital signal-processing system (CED Power 1401 and Spike 2 program). Field potentials were averaged and amplitude and latency were measured with this system.

**Somatosensory mapping**

The somatosensory mechanoreceptive representation (S1) was mapped in order to determine its positional relationship with the vagal evoked potential focus. Since the detailed S1 map of the trigeminal intraoral region has been established (Taira 1987), it was sufficient to examine the receptive fields of middle layer neurons at several arbitrarily chosen sites to extrapolate the location and extent of S1. After the surface and depth recordings of the vagal potential were completed, the dura was further opened widely to expose S1 posterior to the coronal sulcus. The microelectrode was inserted to a depth of ~1 mm and multi-unit responses were recorded from the middle cortical layers in response to natural tactile stimulation of the head, oral and forelimb regions. The stimuli were tapping, brushing, or rubbing the skin and intraoral structures, and
moving the jaw, forelimb, and toes, in order to stimulate all available mechanoreceptors, including in the pharynx and nares. Receptive fields were recorded on standard drawings.

**Histology**

During the microelectrode recordings, several penetrations were marked with lesions made by cathodal current (~15-30 µA for 30 sec). In the macroelectrode recordings, several recording sites were marked with dye deposite (Pontamine skyblue, ~100 µA for ~1 min under microscopic observation) at the termination of the experiment. After the experiment the animals were injected with a lethal dose of barbiturate and the cortex was removed. The tissue block was soaked in 10% Formalin for one week, then in 30% sucrose in Formalin for one week, and then cut in serial 60 mm coronal frozen sections and stained with thionin. Cytoarchitectonic areas were determined using criteria based on Avendano and Verdu (1992), Ghosh (1997), Hassler and Muhs-Clement (1964) and Leclerc et al. (1994). Digital photomicrographs were taken with a Leaf Microlumina, and the images were processed with Adobe Photoshop for adjustment of brightness and contrast as well as insertion of indications and anatomic landmarks.

**RESULTS**

**Macroelectrode surface map**

Vagal stimulation produced a biphasic positive-negative potential over a broad region of the sigmoid, coronal and orbital gyri in chloralose-anesthetized animals. The field was quite broad in chloralose-anesthetized cats but more focused in Saffan-anesthetized cats (see Discussion). Even though the response was diffuse, there were two sites with larger response amplitude than neighbors: one in the anterior part of the lateral sigmoid gyrus, and another more posterolaterally in the orbital gyrus. The former region is the subject of the present study, whereas the latter corresponds to the well-established "insular visceral sensory cortex" (Clasca et al. 1997). Stimulation of either right or left vagus nerves elicited the evoked potential in the sigmoid gyrus.
The contralateral (right) nerve or the ipsilateral (left) nerve was primarily used to examine the evoked potential with macroelectrode recording in three cats or one cat, respectively, and in the other three cats the evoked potentials both sides were examined. No systematic difference was observed in the focal sites or amplitude related to the laterality of stimulation (see also Aubert and LeGros 1970).

The evoked potential on the sigmoid gyrus was focused near the anterior (lateral) tip of the coronal sulcus. Fig. 1 shows a surface map of this potential field in a cat anesthetized with Saffan. The potential distribution was centered just anterior to the tip of the coronal sulcus. The gradient of the potential decay with distance from the focal site was relatively weak and the potential field extended widely both medially and laterally to the sulcus. However, the potential amplitude was generally greater in the region medial to the sulcus than laterally, and it was clearly focused at a site anteromedial to the tip of the sulcus. In fact, in every animal (n=7) the macroelectrode focal site was just medial to the anterior tip of the coronal sulcus, and no other potential focus was evident in the region just lateral to the coronal sulcus.

Histological examination of sites marked with dye following macroelectrode surface mapping in two cats showed that the center or the largest response site of the evoked potential region appeared to be in area 3a (arrowhead, Fig. 1C), characterized both by a well developed granular layer and large pyramidal cells in the fifth layer. This is consistent with the general concept in prior histological studies (Avendano and Verdu 1992; Hassler and Muhs-Clement 1964) that the cortex adjacent to the anterior tip of the coronal sulcus, including both medially and laterally adjacent regions, consists of area 3a.

**Microelectrode surface map**

The vagal responsive field examined with macroelectrode recordings from the intact dura was broad and poorly focused. By contrast, potential mapping from the pial surface with a microelectrode gave far better localization of the focal area. Figure 2 shows an example of such mapping. Although the surveyed area was smaller than that of Fig. 1, Fig. 2 still shows that
negligible responses were obtained in the periphery of a very clear response focus. The large amplitude evoked potentials were concentrated in a region just larger than 1 mm\(^2\). The difference in amplitude of the recorded potential between the response center and periphery was so dramatic that it was easy to demarcate the focus of the vagal evoked potential region on the cortical surface. With microelectrode mapping it was clearer that the vagal responding region was restricted to the area medial to the coronal sulcus even though it closely adjoined the sulcus.

In four cats anesthetized with Saffan, a complete microelectrode map was recorded from the pial surface with contralateral vagal stimulation. The latency and amplitude of the evoked potential were both relatively invariable between cats; the average peak amplitude was 685 mV (range: 482-780) and the mean value of the peak latency was 11.0 msec (range: 9.4-12.1). Fig. 3 illustrates the distribution of vagal potentials relative to the coronal sulcus in all four animals (Fig. 3A-D). The recording sites were spaced ~0.5 mm in general, and ~0.2 mm at the focal sites. In all four animals, the largest evoked potentials were obtained from a stripe-like region, rather than a focal concentric region, and they were restricted to a small region anteromedial to the coronal sulcus. Slight variations in peak amplitude at adjacent points in the focal site were ascribed to variability in the contact pressure of the microelectrode due to surface movement and dimpling.

**Somatosensory mapping**

With the vagal evoked potential focus well localized on the anteromedial side of the anterior tip of the coronal sulcus, we investigated its relationship with the physiologically determined S1 somatosensory representation in three cats by making depth recordings with the microelectrode. The region surrounding the anterior part of the coronal sulcus was mainly examined in accordance with the maps produced by Felleman et al. (1983) and by Taira (1987). Fig. 4 shows two examples. In Fig. 4A, the most anterior mechanoreceptive responses were obtained with stimulation of the ipsilateral anterior tongue (site 1). More posteriorly, receptive fields on the contralateral hard palate (site 2), lower teeth (site 3), upper gingiva (site 4) and lip (site 5) were
found in this order. Further posteriorly, extraoral receptive fields were found, such as the subnostril regions (sites 6, 7). In the case illustrated in Fig. 4B, the most anterior responsive site was found with bilateral glottal stimulation (site 1); more posteriorly, the lip (site 2) and facial responding sites (sites 3-5) were found. Outside these oral/facial region, penetrations encountered neurons with receptive fields on the forelimb (sites 6-8). All but one (site 7) of these sites were located lateral to the coronal sulcus. In the three cats, the most anterior somatosensory representation in the region of the coronal sulcus was the tongue (Fig. 4A, site 1), glottal region (Fig. 4B, site 1) or frontal teeth (not shown). Penetrations were made further anterior to these sites and no further mechanoreceptive responses were obtained. The most anterior mechanoreceptive region was separated by at least 1 mm from the vagal response focus.

On the other hand, multi-unit responses to proprioceptors of the forelimb were found medial to the sulcus (Fig. 4B, sites 6,8), at the antero-posterior level of the facial representation. They responded to claw movement or forepaw tap (cf. Dykes et al 1980). The area just lateral to this deep input region and medial to the vagal site was unresponsive to conventional stimulation, including press/pinch of the contralateral cheek or intraoral palpation. In one animal (Fig. 4A), evoked potentials were obtained in response to stimulation of the ipsilateral masseteric nerve in this region, just anterior and medial to the coronal sulcus. Although the focal site was closer to the vagal site than the forelimb response sites, it was still separate by ~1 mm (posterior and medial) from the vagal evoked potential focus.

Since the cytoarchitectonic location of S1 is well established, we examined histologically only few of the recording sites of somatic receptive fields. They were found in either area 3b or 3a, according to whether surface or deep receptive fields were recorded, respectively, as established in the literature.

**Depth recording**

The same microelectrode used for surface mapping of the vagal evoked potential and for mapping of multi-unit mechanoreceptive responses was also inserted into the cortex in the vagal
response region to determine whether the generator site could be demonstrated by a polarity reversal indicative of the current sink. Since the vagal region was quite well localized, only a few penetrations at the focal site were needed for this purpose. The penetrations were not always perpendicular to the cortical surface, so that even a penetration at the center of the focal site did not always record the deep negative potential with an amplitude comparable to the surface positivity. Nonetheless, in all three animals in which depth recordings were made, deep negative potentials with comparable latency were recorded and reversals were observed in the cortical depth below the surface focal sites.

Fig. 5 shows an example of depth recording at the penetration corresponding to the most posterior and lateral of the focal recording sites indicated by large dots in Fig. 3A (open arrow). The evoked field potential was recorded at five different depths, and a lesion was made at the deepest point (Site 5). The surface positive potential became larger in the middle of the track (sites 2 and 3, corresponding to layer III of the cortex), and it reversed to a negative potential in the depth (sites 4 and 5, layers IV and V/VI border, respectively). Fig. 5C also shows a multi-unit response recorded at site 2; many spikes responded to the stimulation, with latencies between 8 and 22 msec, consistent with the onset and peak latencies of the field potential recorded at the same site of 5.8 and 10.4 msec, respectively. Multi-unit responses to vagal stimulation and polarity reversals of the evoked field potential were obtained in all three depth recordings (open arrows) located at the anterior, middle and posterior parts of the surface vagal focus in this particular animal. Multi-unit responses in penetrations with polarity reversals were also obtained in one other cat. These results definitely demonstrated that there was a cluster of neurons activated at short latencies by vagal input in the center of the response focus determined by the surface mapping.

Cytoarchitecture

Fig. 5D shows the entire electrode track for the penetration just described, from the entry (arrow) to the lesion (double arrowhead), in a Nissl-stained section. In this photomicrograph, the
area demarcated by the four open arrows is recognizable as area 3a. Area 3a was most clearly
distinguished when situated as the transitional area between areas 4 and 3b at more posterior
levels (cf. Fig. 7). Area 4 was characterized by both the lack of layer IV and the presence of the
dark-stained giant pyramidal cells in layer V. By contrast, area 3b was characterized by a thick
layer IV and a cell-sparse stripe-like layer V. Area 3a was distinguishable as an area intercalated
with these two areas, with clear but less thick layer IV overlying giant pyramidal cells in layer V.
Area 3a was also distinguishable from areas 3b and 4 by the sub-lamination of layers III and VI
in the latter two areas. At more anterior levels, such as Fig. 5D, area 4 was replaced by area 6,
which made the medial border of area 3a less clear. However, by tracing anterior from the more
easily distinguished posterior levels, it was possible to follow and delineate area 3a with its
characteristically well-developed granular layer and darkly stained large pyramidal cells in layer
V. The penetration shown in Fig. 5D was entirely within the limits of area 3a. That is, the large
positive potential in the upper layers, the smaller negative potential in the deeper layers, and the
multi-unit responses in the middle layers were recorded in area 3a. In all four brains in which
microelectrode mapping and depth recordings were made, large positive supragranular potentials
and negative infragranular potentials were recorded within area 3a, as histologically verified.

Fig. 6 shows the deep field potential recordings in another case, in which penetrations were
made at the two sites indicated by arrows in Fig. 3C. In this cat, the penetrations were nearly
normal to the cortical surface. Fig. 7 shows the reconstructed cytoarchitectonic limits of area 3a
surrounding two microelectrode penetrations across a series of consecutive sections spaced 120
µm apart. The lesions marked in Fig. 7 were made at the approximate depth of the reversal of the
field potential in each penetration. The lesions are evident in the accompanying
photomicrographs of the indicated sections. Both penetrations were, like that shown in Fig. 5,
just medial to the anterior tip of the coronal sulcus. This part of the cortex was occupied entirely
by area 3a; the lateral border of area 3a with area 3b was at almost the same location in the
sulcus throughout this level. The medial border of area 3a shifted significantly along the antero-
posterior sequence. Posteriorly, the cortex medial to the coronal sulcus included, from medial to
lateral, areas 4, 3a and 3b, and area 3a was wide at this coronal plane. On the other hand, anteriorly, as described earlier, area 4 was replaced by area 6 which extended ventrally, and area 3a became restricted to a narrow region just above the coronal sulcus or the dimple of its anterior tip. Thus, the location of the two penetrations differed with respect to the extent of area 3a, though they were similar in position relative to the coronal sulcus.

As apparent in Fig. 7 (B and C), both the entry point and the lesion of both penetrations were within area 3a. Since these two penetrations were made at the anterior and posterior parts of the surface potential region, these two sections probably correspond to the anterior and posterior limits of the vagal potential generator area in this brain. The anterior section was at almost the anterior limit of area 3a; in the section only 360 µm anterior, area 3a was no longer detectable. Thus, comparison with the extent of the vagal focal site in Fig. 3C suggests that, in this cat, the vagal afferent region occupied almost all of the most anterior (and lateral) part of area 3a, and it extended posteriorly in the most lateral (ventral) part of area 3a.

DISCUSSION

Our results indicate that the vagal input to sensorimotor cortex is located in area 3a. The vagal input in this region thus resembles deep somatic input and must be considered as part of a different cortical subsystem than the cutaneous part of sensorimotor cortex, or S1. The classical sensory homunculus, based on stimulation-evoked sensory paraesthesia (Penfield and Rasmussen 1950), suggested that visceral organs are represented in the primary somatosensory cortex in the same manner as the cutaneous body surface. Early findings in cats (Aubert 1970; Siegfried 1961) and more recent findings in rats (Ito 2002) seemed to support that scheme, but the present observations in the more differentiated cortex of cats do not support that view. Because areas 3a and 3b are distinguished in the sensorimotor cortex of primates similarly to cats, the same visceral cortical organization could be present in primates, including humans.


**Technical considerations**

We mapped the evoked field potential. A field potential study is advantageous for mapping a broad region because it examines mass activity. Possible contamination by far field potentials (Dong and Chudler 1984) was mitigated by using subdural recording with a high impedance electrode. The response field was in each case restricted and focused, with a steep amplitude gradient around the focal site (Fig. 2) that made it relatively easy to demarcate. Although it lay adjacent to the coronal sulcus, the vagal response focus was not buried in the sulcus. The depth recordings made at the focal sites demonstrated phase reversal, a criterion for the presence of a current sink or generator site within the cortex. Clusters of neurons that responded at comparable latency indicated that the potentials represented neuronal activation at the site. Therefore, the focal sites revealed with microelectrode mapping apparently represent dense vagal afferent input at the potential generating region.

The stimulating electrodes were embedded among neck musculature, so that any stimulus spread would have involved the neck muscles. The short latency of our vagal potential is compatible with neck muscle-evoked potentials. However, the neck muscles are innervated by cervical spinal nerves and consequently represented in the posterior sigmoid gyrus (Barbas and Dubrovsky 1980), posteromedial to the vagal focal region. We did not observe any potential foci in that region during macroelectrode mapping. Furthermore, we stimulated the masseteric muscle at the lateral side of the cranium, which is even more anterior in the body, and its evoked potential site was distinctly posterior and medial to the vagal evoked potential site. Thus, it is unlikely that the present results included responses originating from afferents from surrounding muscles.

We used two anesthetics, chloralose and Saffan. We initiated this study with chloralose because it had been used in previous studies of vagal evoked potentials (Aubert 1970; Korn and Massion 1964; Massion et al. 1966). However, under chloralose the vagal evoked potential field was large and difficult to delimit, just as somatic evoked potentials are (Harding et al 1979). Under Saffan anesthesia, the vagal evoked potential had a comparable peak amplitude but a more
delimitable focus (Fig.1). In addition, Saffan has less suppressive effects on autonomic function than chloralose (Child et al. 1972; Ness and Gebhart 1988; Timms 1981), and it does not produce the variable late evoked potential components that can confound studies under chloralose (Boissonade and Matthews 1993). Thus, in contrast to prior studies of vagal evoked potentials under chloralose or pentobarbital, we found consistently short response latencies under Saffan (see following text).

Composition of the vagus nerve: a mixture of “visceral” and “somatic” afferents?

While the cardio-pulmonary and sub-diaphragmatic branches of the vagus consist entirely of afferents which innervate visceral organs that contain smooth muscle, the cervical vagus nerve that we stimulated contains in addition afferents which innervate oropharyngeal tissues that are characterized by mixed smooth and striate muscle and so could be regarded as “somatic”. In particular, it contains afferents from the recurrent laryngeal nerve that innervate the pharynx, larynx, trachea and upper (distal) esophagus. Our vagal stimulation must have activated these afferents simultaneously, and consequently, the cortical potential we recorded probably represents activation of both oropharyngeal afferents as well as afferents from cardio-pulmonary and sub-diaphragmatic visceral tissues. The vagal evoked potential we recorded over the anterior sigmoid gyrus (sensorimotor cortex) has essentially the same latency and shape as the potential that can be recorded over the orbital gyrus (insular cortex; see Aubert 1970; Korn and Massion 1964; Massion et al. 1966), consistent with the view that similar or identical sets of afferents contribute to both projection sites. A subsequent analysis of the sensorimotor cortical evoked potential from cardio-pulmonary and sub-diaphragmatic vagal afferents could directly validate this view; however, several additional considerations support the inference that the evoked potential we recorded in the anterior sigmoid gyrus includes activation by cardio-pulmonary and sub-diaphragmatic vagal afferents and can be regarded as a putative visceral activation site. First, the evoked potential focus we identified lies in area 3a, not 3b, indicating that it is not part of the mechanosensory (“somatic”) afferent representation in cortex. Second, it lies at the lateral
part of the sensorimotor cortical strip, which is consistent with the activation observed in human cortex by natural stimulation of both the upper esophagus (which contains both striate and smooth muscle layers) and the lower (proximal) esophagus (which contains only smooth muscle), as well as by distension of the stomach and the colon (Aziz et al. 1997, 2000b; Binkofski et al. 1998; Furlong et al. 1998; Hecht et al. 1999; Ladabaum et al. 2001; Lotze et al 2001). This area appears to be homologous to the site we identified in cat (see below). Third, a recent study suggests that the conscious sensation produced in humans by distension of the upper esophagus, despite its potential designation as “somatic”, differs significantly from the sensation produced by stimulation of the overlying chest wall, in that it is diffuse, poorly-localized, and distinctly unpleasant, consistent with the sensations elicited from other “visceral” organs (Strigo et al., 2001). Fourth, single-unit recordings in the region of the vagal evoked potential site in sensorimotor cortex of the rat directly indicate activation by afferent input from viscera, consistent with this inference (Hanamori et al. 1998; Zhang and Oppenheimer 1997). Finally, the vagal afferents that innervate the upper trachea and esophagus have been regarded by some as “somatic” in part because they terminate in the marginal layer of the trigeminal subnucleus caudalis and the upper cervical dorsal horn (Contreras et al. 1982; see also Panneton 1991); however, that pattern of termination for visceral afferents is also present in the spinal cord, where afferents that parallel sympathetic efferent fibers similarly terminate in lamina I of the superficial spinal dorsal horn. Therefore, whereas the following comments refer empirically to the ‘vagal evoked potential site’ in sensorimotor cortex, we infer that this represents a putative visceral afferent activation site.

**Comparison with prior vagal evoked potential studies in cats**

Our results confirmed and expanded the results of prior studies regarding the vagal response focus in the sensorimotor cortex in cats. This site corresponds to Siegfried's (1961, 1962) “area A”, in contrast to “area B” in the orbital gyrus (insular cortex). Subsequent studies (Aubert 1970; Aubert and Legros 1970; Korn and Massion 1964; Massion et al. 1966) generally located the
region as lateral, instead of medial, to the anterior tip of the coronal sulcus in their summary diagrams, though they described the area as between the anterior end of coronal sulcus and the presylvian sulcus just as Siegfried did (1961). Our surface mapping always included the area lateral to the coronal sulcus but never identified a potential focus there, and the focal site was anteromedial to the coronal sulcus in all animals. Furthermore, in none of the prior studies did the actual mapping data show the focus lateral to the sulcus, and at least one figure (Fig. 6 of Aubert and Legros 1970) clearly illustrated the focal site anteromedial to the tip of the coronal sulcus, which is consistent with our observations. Therefore, despite the discrepancy with their summary statements, it is apparent that the vagal response focus we identified is the same as that observed in the prior studies and identified first as area A by Siegfried (1961).

The prior studies detailed the latencies of early and late vagal evoked potentials. The latency of the peak response in the present study (9.4-12.1 msec) was comparable to that of the earliest response in the prior studies (8-25 msec, Aubert 1970; Aubert and Legros 1970; Korn and Massion 1964; Massion et al. 1966), which is attributable to myelinated vagal afferents. One study reported that both fast and slowly conducting myelinated fiber groups contribute to the evoked potentials in both the sensorimotor cortex and the insular cortex (Massion et al. 1966). Potentials at latencies attributable to unmyelinated vagal afferents were not clearly distinguished in the present nor in the prior studies. Unmyelinated C-fibers may be too variable (dispersed) in their conduction times to form a coherent, observable evoked waveform (Porter 1963), or they may be more susceptible to anesthetic than myelinated afferents (cf. Kalliomaki et al. 1993), although vagal C-fiber responses have been observed in rat insular cortex (Ito 1994).

**Comparison with prior vagal evoked potential studies in other species**

In addition to cats, a vagal response focus has been observed in the most lateral portion of sensorimotor cortex in rats, goats, and monkeys (Ito 2002; O’Brien et al. 1971; Siegfried 1962). As in Penfield’s homunculus in human cortex, the vagal area was generally located lateral to the intraoral representation in each of these species. This correspondence suggests that the
sensorimotor vagal area may be homologous throughout mammals. Few studies have examined cytoarchitecture.

In rats, the vagal area was shown to occupy parietal "granular" cortex, implying that it is within S1 (Ito 2002). However, the differentiation of sensorimotor cortex in the rat is primordial, and there is overlap between the regions that receive input from cutaneous and deep receptors (Gioanni 1987). That is, there is not a clear distinction between areas 3a and 3b in the rodent. Although it has been suggested that the transitional dysgranular part of S1 in rats receives deep input like area 3a of more encephalic animals (Chapin and Lin 1984), this region was identified only in the spinal representation. Thus, the location of the vagal region in the granular cortex lateral to the trigeminal region in the rat does not necessarily contradict the localization of the vagal region in area 3a in the more differentiated cortex of other mammals.

In macaque monkeys, the vagal evoked potential was recorded in the inferior precentral region at the lateral extreme of sensorimotor cortex (O’Brien et al. 1971). Convergent responses to laryngeal and tongue stimulation were obtained in the same region, anterior to S1, so the authors viewed this region as motor cortex (O’Brien et al. 1971). However, others have regarded the inferior precentral cortex as area 3 extending anterior and lateral from the tip of the central sulcus and providing either the sensory representation of the ipsilateral intraoral region (Manger et al. 1996) or a taste-related region (Benjamin et al, 1968; Ogawa et al. 1985, 1989; Pritchard et al. 1986). Thus, the possibility remains that the vagal potential site in monkey sensorimotor cortex is also located in area 3a.

In humans, direct electrical stimulation of the lateral S1 region elicited conscious alimentary sensation (Penfield and Rasmussen 1950). Esophageal (Aziz et al. 1997, 2000b; Binkofski et al. 1998; Furlong et al. 1998; Hecht et al. 1999), gastric (Ladabaum et al. 2001) or rectal (Lotze et al. 2001) distension elicited activation in functional imaging studies in the same general region around the lateral end of the central sulcus. The pathway responsible for this cortical activation, and whether it represents motor or sensory activity, has not been determined. Although these alimentary structures are dually innervated by cranial and spinal nerves (Collman et al. 1992), a
vagal contribution is strongly suggested by the sequence of cortical topography, consistent with the present findings.

Thus, in all representative mammals that have been investigated, an area at the lateral end of sensorimotor cortex has consistently been observed that could represent vagal afferent activity. However, the cytoarchitectonic location of the vagal response site has not been previously resolved.

**The cytoarchitectonic location of the vagal response focus**

The present results indicate that the region responsive to vagal input is entirely outside the S1 representation, including the representation of the intraoral structures. The somatosensory map of the cat S1 along the coronal sulcus is well established (Felleman et al. 1983; Taira 1987). The most anterior and lateral portion represents the ipsilateral and contralateral intraoral mechanoreceptors, and then the lips and extraoral structures are represented progressively more posteriorly and medially (Taira 1987). Our recordings in S1 reproduced this pattern, and furthermore, we found pharynx- and glottal-responding neurons anterolateral to the representation of the tongue (Fig. 4B). Nonetheless, the vagal responsive region was distinctly anterior and medial to the S1 representation, and a region with no somatic response was intercalated between the vagal region and the S1 representation.

The vagal response focus was anterior and medial to the anterior tip of the coronal sulcus, and cytoarchitectonic studies and our observations indicate that it lies within area 3a. Indeed, Hassler and Muhs-Clement (1964) showed the anterior and lateral border of area 3a crossing the coronal sulcus to include even the region lateral to the anterior part of the coronal sulcus. Avendano and Verdu (1992) construed this lateral extension only at the most anterior tip of the coronal sulcus. Our reading of the present material was even more conservative, and area 3a appeared to be restricted entirely to the medial bank of the coronal sulcus and the lateral sigmoid gyrus.

Lesions made at microelectrode penetrations in which field potential reversals and multi-unit responses were observed were all histologically identified within area 3a. This indicates that the
current sink producing the surface positive potential is definitely located in area 3a, not in area 3b. Although depth recordings were made only in the neighborhood of the focal site, there is no reason to expect additional generator sites which are not revealed by surface potentials. It is possible that vagal input may also activate neurons outside the borders of area 3a, but the main focus is certainly within area 3a. At the anterior end of area 3a where it is narrow (Figs. 5 and 7B), all of area 3a appears to be occupied by the vagal responsive region. More posteriorly, at the level of Figure 7C, the vagal responsive region is restricted to the most lateral part of area 3a. Thus, the present observations indicate that the vagal response focus occupies the most anterolateral part of area 3a in the cat.

**Input source**

Area 3a in cat receives its principal proprioceptive and vestibular inputs from the thalamic region that lies along the border between the ventral posterior and the ventrolateral nuclei, that is, between the lemniscal somatosensory relay and the cerebellar motor relay (Dykes et al. 1986; Jones and Porter 1980; Kaas 1993). Activation of Group I inputs, for example, evokes in area 3a a so-called thalamocortical primary response with an initially positive surface wave which reverses polarity in deep layers (Landgren and Sifvenius 1969; Odkvist et al. 1975). Iwata et al. (1985) described a masseteric nerve evoked potential, which reversed to a deep negative component at a depth of 1.5-2.5 mm, in the rostral part of cat area 3a, consistent with our observations. This depth profile is in good agreement with that of the present vagal potential, consistent with the hypothesis that the vagal input to area 3a arrives by a direct thalamocortical projection like the proprioceptive or vestibular input. The presence of sacral visceral (pelvic nerve) input to neurons in the ventral periphery of the thalamic ventral posterior complex (Brüggemann et al. 1994), from which projections to area 3a also arise (Craig and Kniffki 1985), supports this possibility as well.

The anterolateral region of area 3a receives a massive input from the (visceral) granular insular cortex (Claska et al. 2000). However, that projection terminates in layers I, III and VI, which differs from the middle layer focus of the vagal evoked potential that we observed. In addition,
previous work demonstrated that the rat S1 vagal potential is independent of the insular input (Ito 2002). Thus, at present it seems likely that the vagal input to area 3a ascends by a direct thalamic pathway. Retrograde labeling data are needed to address this issue.

Because activity from the chorda tympani and the vagus nerves are similarly processed in the brainstem (Nomura and Mizuno 1981, 1983), the cortical projection of the chorda tympani, the main taste nerve, must be compared with that of the vagus. Like the vagus, the chorda tympani activates multiple sites in the cortex of both cat and monkey. In the cat, chorda tympani evoked potentials were reported in the orbital gyrus and the coronal gyrus (Burton and Earls 1969), and in the monkey, in the inferior precentral area of the sensorimotor cortex and the opercular/insular cortex (Benjamin and Burton 1968; Benjamin et al. 1968; and Ogawa et al. 1985). The orbital / insular area receives input from the thalamic taste area (monkey: Pritchard et al. 1986; cat: Ruderman et al. 1972; Yasui et al. 1987) and is regarded as primary gustatory cortex. In the monkey, a collateral (“sustaining”) projection from the thalamic taste relay to the sensorimotor cortical areas was proposed (Benjamin and Burton 1968; see Pritchard et al. 1986), though in the cat, taste neurons were not observed in the coronal region (Cohen et al. 1957), and instead, anterograde tracing indicated projections from the thalamic taste relay to orbital, perirhinal and infralimbic cortices (Yasui et al 1987). Thus, the data indicate that the multiple thalamocortical projections of the chorda tympani parallel those of the vagus; however, it remains to be determined whether the vagal and gustatory inputs to the orbital / insular and sensorimotor cortices ascend from common sets of thalamic neurons.

**On the possible role of area 3a in visceral motor and sensory functions**

Stimulation in the somatic portion of area 3a evokes striate muscle contractions (Preuss et al. 1996; Widener and Cheney 1997; Wu et al. 2000), and stimulation of this region can also have autonomic effects. In cats, it can change respiratory rhythm (Kaada 1951), via the phrenic as well as the recurrent laryngeal nerves (Bassal and Bianchi 1981, 1982). Thus, area 3a appears to participate in visceral motor control via both sympathetic and parasympathetic pathways, as well
as in somatic motor control (Jones and Porter 1980; Wiesendanger 1982). Stimulation in the lateral precentral region of the monkey cortex evokes swallowing (Martin et al. 1999) or changes in heart rate (Hast et al. 1974), though whether that region is cytoarchitectonically area 3a remains to be resolved.

It has not yet been determined whether area 3a is involved in the conscious perception of any sensory event. Activity in muscle afferents is essential for the sense of joint position (Goodwin et al. 1972; Gandevia 1985; Clark et al. 1985), so area 3a, as the primary cortical target of deep receptor afferents, may be involved in this sensation (Jones and Porter 1980), though it is heavily interconnected with area 2 posterior to S1 (Porter, 1991). As part of the same cytoarchitectonic area, the vagal afferent projection field in the lateral portion of area 3a could share a role with the insular cortex in sensations such as ‘sinking feeling,’ ‘sick feeling,’ ‘choking sensation,’ ‘nausea’ and ‘shaking in the heart,’ descriptors evoked by direct electrical stimulation of the corresponding human cortex (Penfield and Rasmussen 1950).

**A cortical homeostatic afferent network**

In addition to the region at the lateral end of sensorimotor cortex, two other cortical regions are activated by vagal afferent input, namely, the insular and the cingulate cortices, which also receive vagal input by way of a direct thalamic pathway (Cechetto and Saper 1987; Bachman et al. 1977; Hallowitz and MacLean 1977). The insular cortex has direct interconnections with both area 3a and with the cingulate cortex (cat: Clasca et al. 2000). These three regions apparently constitute the main visceral afferent activation sites in the cortex and form together a visceral afferent cortical network. This interpretation is supported by the consistent activation of all three areas in imaging studies of visceral stimulation in the human brain (Aziz et al. 2000a). Notably, the same three regions (area 3a, the insular and cingulate cortices) also constitute the main regions activated in the human cerebral cortex by painful stimulation (see Craig, 2002), and prior evidence in the cat indicates that area 3a also receives nociceptive thalamocortical input (Craig and Kniffki, 1985). Thus, the present findings are consonant with the fundamental concept that
pain and visceral sensation are different aspects of a common homeostatic afferent system that activates limbic sensory (insular) cortex, limbic motor (cingulate) cortex and a particular portion of sensorimotor cortex (area 3a) (Craig, 2002). The role of area 3a in this network remains to be resolved.

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FIGURE LEGENDS

Fig.1 Epidural surface map made with a macroelectrode. A. Lateral view of the cat left cerebral cortex indicating the location of the coronal sulcus. B. A vagal evoked potential map at points spaced ~1 mm along orthogonal co-ordinates showing a regional focus anteromedial to the tip of the coronal sulcus. C. A photomicrograph of a coronal section showing a dye mark (double arrowhead) made at the center of the responsive region. Open arrows delimit area 3a. Cru: cruciate sulcus. Cor: coronal sulcus. Pre: presylvian sulcus.

Fig.2 Pial surface map made with a microelectrode. A. Photograph of the mapped region at the anterior tip of the coronal sulcus. Dots indicate recording sites. Cru: cruciate sulcus. Cor: coronal sulcus. B. Focused map of vagal evoked potentials obtained at the sites indicated in A. These data are from a different animal than those in Fig. 1.

Fig.3 Distribution of vagal evoked potentials in four (A-D) surface microelectrode recording experiments. In each figurine the dots indicate the amplitude of the evoked potential at each recording site, plotted relative to the anterior tip of the coronal sulcus (Cor), according to the sizes in the legend. The length of directional arrows corresponds to 1 mm. The open arrows in A and C indicate the sites where depth recordings were made; the recordings at the most caudal arrow in A and the two arrows in C are shown in Figures 5 and 6, respectively, and the corresponding histological photomicrographs are shown in Figures 5 and 7.

Fig. 4 Location of vagal evoked potential foci with respect to S1 cortex, identified by depth recordings of somatosensory responses. In both examples (A and B), the multi-unit receptive fields at the numbered symbols along the coronal sulcus (Cor) were found at the sites indicated by the numbers on the drawings. Filled dots indicate tactile responses, open circles indicate
proprioceptive responses, and open triangles indicate strong vagal evoked potentials. Activation from the forepaw occurred by mechanoreceptors on the glabrous skin of the digit (7) or movement of the claw (6, 8).

Fig. 5 Demonstration of microelectrode depth recording at the vagal response focus. This penetration was made at the surface site marked with the caudal open arrow in Figure 3A. A. Reconstruction of the electrode track on a line drawing of the cortical section. Cru: cruciate sulcus. Cor: coronal sulcus. Pre: presylvius sulcus. The labels IV and V indicate the fourth and fifth layers, respectively. Open arrows demarcate the cytoarchitectonic borders of area 3a. The numbers along the track indicate the sites of the recordings shown in B. C. Multi-unit activity recorded at site 2. D. Photomicrograph of the corresponding coronal section. Open arrows indicate cytoarchitectonic borders. Filled arrow indicates the entry point and direction of the penetration, and the double arrowhead indicates the lesion made at the bottom of the track. Roman numerals indicate cortical layers.

Fig. 6 Microelectrode potentials recorded at different depths in penetrations at the two sites indicated in Figure 3C, showing reversal in the middle layers 900-1200 µm deep in each penetration.

Fig. 7 A summary of the cytoarchitecture of the vagal response region in one reconstructed case. A series of drawings of coronal sections at 120 µm intervals extending rostral (left) to caudal (right) from the experiment shown in Figure 3C. The solid lines indicate the borders of area 3a, and the dots indicate lesion sites. B and C show photomicrographs of the indicated sections containing the lesions (arrowheads); the open arrows indicate the borders of area 3a. Dorsal up, lateral right.
Figure 2
Figure 3
Figure 4
Figure 6