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Somatosensory and multisensory properties of the medial bank of the ferret rostral suprasylvian sulcus

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Abstract

In ferret cortex, the rostral portion of the suprasylvian sulcus separates primary somatosensory cortex (SI) from the anterior auditory fields. The boundary of the SI extends to this sulcus, but the adjoining medial sulcal bank has been described as “unresponsive.” Given its location between the representations of two different sensory modalities, it seems possible that the medial bank of the rostral suprasylvian sulcus (MRSS) might be multisensory in nature and contains neurons responsive to stimuli not examined by previous studies. The aim of this investigation was to determine if the MRSS contained tactile, auditory and/or multisensory neurons and to evaluate if its anatomical connections were consistent with these properties. The MRSS was found to be primarily responsive to low-threshold cutaneous stimulation, with regions of the head, neck and upper trunk represented somatotopically that were primarily connected with the SI face representation. Unlike the adjoining SI, the MRSS exhibited a different cytoarchitecture, its cutaneous representation was largely bilateral, and it contained a mixture of somatosensory, auditory and multisensory neurons. Despite the presence of multisensory neurons, however, auditory inputs exerted only modest effects on tactile processing in MRSS neurons and showed no influence on the averaged population response. These results identify the MRSS as a distinct, higher order somatosensory region as well as demonstrate that an area containing multisensory neurons may not necessarily exhibit activity indicative of multisensory processing at the population level.

Keywords

Tactile; Auditory; Crossmodal; Somatotopy; Map

Introduction

It is well established that the mammalian neocortex contains separate, spatially distinct representations of the somatosensory, auditory and visual sensory modalities. Although the cores of each of these different areas are, for the most part, functionally and connectionally distinct, a recent focus of investigational effort has been directed toward examining the nature of common borders between representations of different sensory modalities. For example, in the cat cortex, the visuotopically organized postero-lateral lateral suprasylvian (PLLS; Palmer et al. 1978) area shares a border with the dorsal zone (DZ; Stecker et al. 2005) of auditory cortex. Recording penetrations across this border reveals the presence of neurons responsive to one modality and then the other, separated by a band of neurons responsive to both (Allman and Meredith 2007). Furthermore, because both PLLS (Rauschecker et al. 1987) and DZ

(Lomber et al. 2008) are involved in processing of stimulus motion, and the bimodal border neurons demonstrate visual receptive fields in the periphery of the PLLS visuotopic representation (Allman and Meredith 2007), it seems possible that the multisensory properties of these regions are not generalized crossmodal effects, but are related to the functional roles of the regions in which they reside. Thus, the organization of cortical sensory and multisensory properties may not so much resemble core/matrix patches as modules in which different sensory inputs provide the substrate underlying a specific behavior or perception. Further support is necessary to test this hypothesis, and the present study was initiated to examine the shared border between somatosensory and auditory representations in the ferret cortex.

In ferret cortex, the posterior portion of the rostral suprasylvian sulcus separates primary somatosensory (SI; McLaughlin et al. 1998; Rice et al. 1993) cortex from auditory (AVF, ADF, AAF; Bizley et al. 2005; Kowalski et al. 1995) cortices, as illustrated in Fig. 1. Here, the medial and lateral banks of the rostral suprasylvian sulcus intervene between the two representations and might potentially represent a multisensory transition from one region to the other. A similar region of the rostral suprasylvian sulcus in the cat is included in the traditionally defined multisensory “suprasylvian fringe” (Heath and Jones 1971a, b) that separates auditory (AAF: Reale and Imig 1980) from somatosensory gyral cortices (area 5: Avendano et al. 1988; Graybiel 1972; Hassler and Muhs-Clement 1964) and the third somatosensory region, SIII (Dykes et al. 1977; Tanji et al. 1978). Recent examination of the lateral bank of the suprasylvian sulcus in cat, where it abuts the auditory AFF, has revealed that there are transitions between the auditory and somatosensory representations that contain multisensory (auditory–somatosensory) neurons (Clemo et al. 2007; Monteiro et al. 2003). In the ferret, a preliminary examination of the lateral bank of the rostral suprasylvian sulcus suggests that it is a site of multisensory convergence (Keniston et al. 2008). However, recordings from the medial bank of the rostral suprasylvian sulcus (MRSS) report that neurons in this region are “unresponsive” to somatosensory stimulation (Rice et al. 1993). Therefore, the present study used electrophysiological and anatomical techniques to examine the MRSS in the ferret with the goal to assess the potential for this region to represent a transition between two modalities located on each side of it. A preliminary abstract of this work has been presented (Clemo et al. 2008a).

Materials and methods

All procedures were performed in compliance with the Guide for Care and Use of Laboratory Animals (NIH publication 86–23) and the National Research Council’s Guidelines for Care and Use of Mammals in Neuroscience and Behavioral Research (2003) and approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University. Most procedures are described in detail in Dehner et al. (2004) and are largely summarized here. A total of ten ferrets were used as detailed below.

Surgical procedures

For electrophysiological recording procedures, ferrets ($n = 5$) were anesthetized with sodium pentobarbital (40 mg/kg i.p.) and surgically implanted with a recording well 3–5 days prior to recording, using aseptic techniques described in detail elsewhere (Allman et al. 2008a).

Electrophysiological recording

For recording, the ferrets first were anesthetized with ketamine/acepromazine (35 mg/kg ketamine; 2 mg/kg acepromazine intramuscularly), and the implanted well secured to a supporting bar. The animals were intubated through the mouth, ventilated (expired CO₂: ~4.5%) and immobilized (pancuronium bromide; 0.3 mg/kg initial dose; 0.2 mg/kg h supplement i.p.). Fluids and supplemental anesthetics (8 mg/kg h ketamine; 0.5 mg/kg h

acepromazine i.p.) were administered continuously with an infusion pump. Animal temperature was maintained at 38°C on a heating pad and body temperature and heart rate monitored continuously. In two animals, to map receptive fields, single unit recordings across SI and MRSS were made using a tungsten electrode (tip exposure ~20 µm, impedance <1 MΩ). In three other animals, analysis of response properties of MRSS neurons was made using a 32-channel silicon probe (1×32–5 mm 100–413 array; impedance ~1 MΩ; NeuroNexus Technologies, Ann Arbor, MI). In either case, the electrode was advanced using a hydraulic microdrive.

Once a neuron was identified, its responses to a wide range of tactile (manually held brush, probe and von Frey hairs as well as joint rotation) and auditory (manually delivered clicks) stimuli were assessed and, when possible, the receptive fields mapped. Somatosensory receptive fields were mapped using minimally effective stimuli (force thresholds determined using calibrated Semmes-Weinstein monofilaments) and graphically recorded on a scaled drawing of the ferret's body surface.

In those cases where the multichannel electrodes were used, the neurons were then quantitatively examined in response to electronically triggered stimuli in one of three combinations: auditory alone, somatosensory alone, or combined auditory–somatosensory. Auditory stimuli (100 ms duration, 81 dB SPL white noise), were generated (RX-6; TDT) using a Gaussian function delivered through FF1 magnetic speakers at 45° azimuth, 0° elevation 35 cm from the animal's head contralateral to the recording site. Somatosensory stimuli were generated by an electronic ramp generator that drove a modified shaker (Ling 102A) with an attached nylon monofilament that delivered a tap (2.5×10^{-2} g force) to the body surface. Each stimulus combination was presented 50 times in random order with an intertrial interval randomized to between 3 and 8 s. Each stimulus was positioned to fall within the receptive fields as many as possible of the neurons within a given penetration. Neuronal responses from each of the recording channels were digitized at 25 kHz using a Tucker Davis neurophysiology workstation (System III, Alachua, FL) and stored on a PC for later analysis. At the termination of the experiment, animals were euthanized (barbiturate overdose) and perfused intracardially with saline followed by fixative. The brain was blocked stereotactically, removed and cryoprotected. Sections (50 µm thick) were cut serially using a freezing microtome, and stained with cresyl violet to histologically reconstruct each electrode penetration.

Data analysis

For analysis of multichannel recording data, individual neuronal waveforms were sorted using an automated Bayesian sort-routine. The responses of each neuron were determined after the method of Bell et al. (2005): response onset was defined as the point at which activity level exceeded three standard deviations above median spontaneous activity with a minimum of 15 ms of activity sustained at that level; response offset was defined as activity that dropped below the sustained response level and remained below it for 15 ms; response duration was defined as the period between the response onset and offset. For each of the three stimulus conditions, the time duration from the earliest onset to the latest offset of any response was used to calculate the spike counts (mean and standard deviation) elicited from the neuron. A paired *t* test was used to assess if each separate-modality response differed significantly from the combined condition.

Based on the suprathreshold responses to auditory or somatosensory stimuli (visual stimuli were uniformly ineffective in this region), neurons were classified as bimodal (responded to both auditory and somatosensory stimuli presented alone) or as unimodal (responded to only one stimulus when presented alone). Neurons classified as unimodal could be further distinguished into one of two categories. Some unimodal neurons had responses that were significantly influenced by the presence of an ineffective stimulus modality, and these neurons

were defined as subthreshold multisensory neurons (after criteria of Allman and Meredith 2007). Unimodal neurons not significantly affected by combined-modality stimulation retained their designation as unimodal neurons. Those neurons that did not meet the mean activity requirement (of 0.5 mean spikes/trial) were regarded as unresponsive. The sensory/multisensory status of each neuron was then tabulated with other response, receptive field and anatomical features for purposes of comparison and analysis.

Neuroanatomical procedures

Ferrets ($n = 5$) were anesthetized with pentobarbital (40 mg/kg i.p.), their heads secured in a stereotaxic frame and, under aseptic conditions, a craniotomy was performed to expose the desired cortical area. Guided by sulcal/gyral landmarks, tracer injections targeted either the MRSS ($n = 3$) or the SI face representation on the suprasylvian gyrus ($n = 2$). An electrode carrier was used to support a 31 gauge, 5 μ l Hamilton syringe containing the tracer biotinylated dextran amine (BDA; 10,000 MW; lysine fixable; 10% in 0.1 M phosphate buffer). The BDA was pressure injected (volume = 0.350–1.8 μ l). For wide cortical areas, multiple injections were made to ensure a representative filling of the region. After completing the injection, the cortex was covered with gel foam, the wound sutured closed, and standard postoperative care was provided.

Following a 7–10-day post-injection survival period, animals were euthanized (barbiturate overdose) and perfused intracardially with saline followed by 4.0% paraformaldehyde fixative. The brain was blocked stereotaxically, removed and cryoprotected. Coronal sections (50 μ m thick) were cut serially using a freezing microtome. One series of sections (usually 250–300 μ m interval) was processed for BDA visualization using the Veenman et al. (1992) protocol, intensified with nickel–cobalt. Reacted sections were mounted on pre-treated slides, dehydrated and coverslipped. An alternate series of sections were counterstained with cresyl violet to help visualize cytoarchitectonic features. In three cases, another series of sections was processed to visualize cytoarchitectonic features using the antibody SMI-32 to non-phosphorylated neurofilaments (van der Gucht et al. 2001).

Tissue sections processed for BDA were examined using a light microscope and the locations of labeled neuronal features were plotted using a PC-driven digitizing stage controlled by Neurolucida software (MBF Biosciences). Each tissue section was traced showing its tissue outline and gray matter/white matter border, upon which the locations of labeled neurons and boutons were plotted. The injection site was defined as the region at the end of the injection track containing a dense aggregation of labeled neuronal somata, processes and neuropil. Retrogradely labeled neurons appeared densely black within their somata and proximal dendrites. BDA-labeled axon terminals appeared as distinct black swellings at the end of thin axon stalks, or as symmetrical varicosities along the length of a labeled axon. Plotted tissue sections from each case were digitally transferred to a graphics program and serially superimposed for final visualization and graphic display. Cytoarchitectonic features visualized using SMI-32 immunohistochemistry were plotted using a camera lucida and photographed.

Results

Cytoarchitectonic features of the MRSS

The MRSS, when reacted for SMI-32, revealed the general laminar organization seen in homotypical sensory neocortex, as depicted in Fig. 2. Perhaps most characteristic of the region was its expanded and densely stained supragranular layers (layers II and III), with layer III being packed with granule cells and small pyramidal cells. In addition, layer IV was sparsely stained whereas the infragranular layers were compressed, with small pyramidal cells and randomly oriented short fibers. These cytoarchitectonic features were quite distinguishable

from those of SI on the suprasylvian gyrus, which exhibited compressed and modestly stained supragranular layers, a wide layer IV, and expanded infragranular layers with many vertically oriented, stained fibers. The cytoarchitectonic transition from SI to MRSS appeared to occur just deep to the lip of the sulcus, where the supragranular layers begin to expand and layer IV becomes narrower and less sparsely stained. Deeper, as the bank transitions into the fundus, the supragranular layers remained dense and well stained while the granular and infragranular layers became greatly compressed, to the point where layer IV was difficult to detect. In the fundus, lower layer III also contained occasional large, well-stained pyramidal neurons. These distinguishing features of the MRSS were observed to extend for approximately 4 mm anterior to the A-P level of the posterior end of the pseudosylvian sulcus (see gray arrow in Fig. 1).

Sensory properties of the MRSS

Multichannel extracellular recordings were made in three ferrets in which recording penetrations ($n = 14$) collectively covered the width and depth of the MRSS. From these efforts, a total of 582 neurons were examined, of which 62% ($n = 363$) were responsive to sensory stimulation. Examples of responses that were encountered are provided in Fig. 3. Traditionally, neurons have been classified by their responses to different sensory stimuli presented independently. Accordingly, neurons activated by a single sensory modality were classified as unimodal. In the MRSS, unimodal neurons represented the overwhelming majority (84%; 304/363) of sensory neurons. Of these, nearly all (98%; 297/304) were activated only by somatosensory cues; a few (2%; 7/304) were activated by auditory stimulation. A modest proportion of neurons (16%; 59/363) were excited by both somatosensory and auditory stimuli presented alone, and these were classified as bimodal. The distribution in the MRSS of neurons showing these types of responses is depicted in Fig. 4, and summarized in Table 1. Somatosensory and bimodal neurons were found throughout the MRSS in all lamina, while auditory cells were infrequent and found only in the supragranular layers.

Somatosensory properties of the MRSS

Neurons (both unimodal and bimodal) responsive to somatosensory stimulation constituted 98% (356/363) of the sensory neurons in the MRSS. The overwhelming majority (98.4%) of these neurons were activated by low-intensity cutaneous (light touch) stimulation corresponding to activation of hair. Somatosensory receptive fields were mapped for 129 neurons from 3 ferrets and the body regions most frequently represented were the head (85%), neck (8%) and adjoining region of the trunk (5.8%). Receptive fields on the forelimb (<1%) were rarely observed, and those on the hindlimb and tail were not found at all. The preference for anterior (e.g., head and neck) receptive fields was similar for both unimodal and bimodal neurons. The majority (56%) of all somatosensory neurons had bilateral receptive fields, the size of which ranged from small, such as those covering both sides of the nose, to large, which included all of the head and upper torso on each side. Comparison of the distribution of bilateral receptive fields among unimodal and bimodal neurons revealed, however, that the proportion of bimodal neurons with bilateral somatosensory receptive fields was much greater (76%) than that of the unimodal neurons (54%).

Although somatosensory receptive fields primarily represented the anterior portions of the body surface, there was evidence for somatotopy within this restricted representation in the MRSS. In each of the cases, neurons with receptive fields on the rostral face and head tended to be located in the outermost portions of the MRSS, while those with receptive fields on the back of the head and torso were identified deeper in the MRSS (see Fig. 5). In addition, receptive fields tended to be somewhat smaller for neurons found anteriorly in the MRSS than for those in more caudal locations. Thus, the receptive fields represented at successively more posterior and deep positions in the MRSS shift from the vibrissal/cheek position on the face to more caudal locations on the head/neck while also progressively increasing in size.

Comparison of somatosensory MRSS with SI

The MRSS lies in the bank of the rostral suprasylvian sulcus that forms the lateral border of SI in ferret cortex (see Fig. 1). As a consequence, many penetrations that targeted the MRSS began by traversing the lateral portion of the SI representation. The majority of sites sampled in the suprasylvian gyrus were activated by cutaneous stimulation of the contralateral face, while a few also included the adjacent neck region, as illustrated for a series of penetrations in a single case in Fig. 6. At rostral sites, receptive fields were small and located primarily on the vibrissal or nose region. At more caudal sites along the gyrus, receptive fields on the head increased in size and shifted to more dorsal and caudal positions on the head. The transition from SI into MRSS was often quite subtle since both these different regions shared several organizational features such as the representation of rostral to caudal regions of the face/head at anterior to posterior sites, respectively. In recording penetrations that extended from SI into the MRSS, typically the SI receptive fields on the face were the smallest on SI, and then increased in size from the outer to deeper regions of the MRSS. However, in contrast to the MRSS, SI neurons displayed receptive fields that were consistently smaller, were rarely bilateral, and were never bimodal (somatosensory–auditory).

Connectivity of MRSS

Tracer injected into the MRSS ($n = 3$) revealed retrogradely labeled neurons primarily in the adjacent portions of SI, as illustrated in Fig. 7a. Neurons projecting to MRSS were found both on the suprasylvian gyrus portion of SI as well as along the lateral bank of the coronal sulcus. These labeled neurons were largely found in layer III, although some of those identified in the coronal sulcus were located in layer V. In addition, a few labeled neurons were observed in the opposite bank of the suprasylvian sulcus, where the lateral suprasylvian sulcal (LRSS; Keniston et al. 2008) multisensory region has been described. It should be noted that no neurons projecting to MRSS were observed on the coronal gyrus (body and limb representation of SI), or in the more posterior somatosensory-related areas of SIII or rostral posterior parietal (Manger et al. 2002) cortex. A few retrogradely labeled neurons were also identified in the banks and fundus of the posterior end of the pseudosylvian sulcus. This pseudosylvian region is known to receive projections from SI (Ramsay and Meredith 2004). Thalamocortical projections to the MRSS (not depicted) almost exclusively arose from the central aspects of the ipsilateral ventrobasilar nucleus.

To confirm the connection between SI and MRSS, tracer was injected into the SI face representation on the suprasylvian gyrus ($n = 2$). As a consequence, orthogradely labeled axons and axon terminals were identified across all cortical laminae from the fundus to the lip of the MRSS, as depicted in Fig. 7b.

Auditory properties of the MRSS

In addition to the unimodal somatosensory neurons that dominate the MRSS, a small population (2%; 7/363) of unimodal auditory neurons was identified (see, for example, Fig. 3b). These auditory neurons responded to contra-lateral acoustic stimulation and appeared to be segregated in their distribution within the MRSS to the supragranular layers (see Fig. 4d). Bimodal neurons (16%; $n = 59/363$; see below) also responded to contralateral auditory stimulation but were distributed throughout the different laminae of the MRSS (Fig. 4e). It should be noted that a fixed stimulus (100 ms, 81 dB SPL, white noise at 45° azimuth, 0° elevation) was used to identify neurons with auditory sensitivity. Because some neurons might prefer other stimulus configurations, it is possible that the incidence of auditory effects in the MRSS might be an underestimation. However, the same auditory stimulus configuration was used in a companion study of the lateral bank of the rostral suprasylvian sulcus (Keniston et al. 2008), which identified a larger proportion of auditory inputs than observed in the MRSS. Therefore, these stimuli appear to be adequate to address issues of proportional representations of sensory

responses. Despite the presence of auditory responses in the MRSS (18%; $n = 66/363$), tracer studies failed to identify a potential auditory source for these inputs, except for the possibility that auditory information may be relayed to the MRSS from auditory/multisensory neurons in the opposite bank, the LRSS (see Fig. 7a).

Multisensory properties of the MRSS

Conventional methods of assessing neuronal multisensory properties define as multisensory those neurons whose activity is *influenced* by more than one sensory modality. The present experiments tested all identified neurons with both separate- and combined-modality stimulation to assess whether the stimuli can excite or influence responses. Using this paradigm to examine MRSS neurons, some (16%; $n = 59/363$) showed traditional bimodal patterns of activity (i.e., were activated by somatosensory and by auditory stimuli presented alone; see Fig. 3c). The majority (66%; $n = 39/59$) of these bimodal neurons had higher discharge levels to somatosensory versus auditory stimulation, and this preference was reflected in the averaged responses of bimodal sample [somatosensory = 11.4 ± 1.2 (mean spikes/trial $\pm$ SEM); auditory = 5.7 ± 0.6]. Figure 8b plots the relationship of responses of bimodal neurons to somatosensory and combined (auditory–somatosensory) stimulation. Surprisingly, only a few bimodal neurons (20%, 12/59) showed a significant response change when the response to a combined stimulus was compared to that evoked by the best unimodal stimulus and, collectively, bimodal neurons generated a slight but significant response increase when presented combined auditory–somatosensory stimuli. It was also striking that the population of bimodal cells had an average level of spontaneous activity [4.01 ± 0.5 (mean spikes/trial $\pm$ SEM)] that was more than double that of any of the other subgroups of neurons in the MRSS as shown in Fig. 8.

This testing paradigm also demonstrated significant multisensory effects in some somatosensory neurons that conventionally appeared to be unimodal. These neurons (13%; $n = 46/363$) showed a significant difference in their response to combined stimulation when compared with that elicited by the somatosensory stimulus presented alone. This subgroup of neurons was termed ‘subthreshold multisensory’ as defined elsewhere (Allman and Meredith 2007; Allman et al. 2008b; Clemo et al. 2007; Dehner et al. 2004; Meredith and Allman 2009) and included neurons in which the tactile response was significantly facilitated ($n = 14$) or significantly suppressed ($n = 32$). Figure 8c plots the relationship of responses of subthreshold multisensory neurons to somatosensory and combined (auditory–somatosensory) stimulation, where it is evident that most examples produced suppressive responses. As also illustrated in that same figure, response activity generated in subthreshold multisensory neurons by somatosensory stimulation was nearly identical to that observed for somatosensory (unisensory) neurons (Fig. 8d), but was significantly less than that observed for bimodal neurons (compare with Fig. 8b). These data are consistent with the likelihood that different groups of multisensory neurons exhibit different functional ranges or modes (see also Perrault et al. 2005). Ultimately, however, the total response of the MRSS to combined auditory–somatosensory stimulation was nearly the same as that elicited by the somatosensory stimulus, as shown in Fig. 8a. Thus, these results support the somatosensory nature of the MRSS as well as demonstrate that an area containing multisensory neurons may not necessarily exhibit activity indicative of multisensory processing at the population level.

Discussion

MRSS: a somatosensory area

Examination of the MRSS demonstrated that this region of ferret neocortex contains a somatosensory representation. Neurons in the MRSS were primarily responsive to low-threshold tactile stimulation activated by hair-type receptors. Their receptive fields varied widely in size, from small areas on the face to large portions of the back of the head, neck and

upper trunk. These features appeared to be arranged in a limited somatotopy of the anterior portions of the body surface (e.g., head, neck, and upper trunk) where small receptive fields on the face were represented anteriorly and on the outer portions of the sulcal bank, while larger receptive fields tended to occur posteriorly and deeper in the sulcus. Anatomically, the MRSS was shown to maintain a reciprocal connection with the head representation of SI.

MRSS and SI

Like the MRSS, the neighboring portion of SI also represents the head. However, the somatosensory properties of MRSS are different from those in SI, since MRSS neurons have receptive fields that are generally larger and bilateral, and can exhibit auditory influences. The transition between the two regions occurs near the lip of the sulcus where the medial bank of the suprasylvian sulcus begins to exhibit an especially fragmented cytoarchitecture quite distinct from that of the adjacent face region of SI (Rice et al. 1993). Thus, these collective observations indicate that the MRSS contains a separate somatosensory representation that is different from SI. Based on these same criteria, the MRSS also appears to be distinct from other somatosensory representations including the third somatosensory area, SIII, and PPr (Leclerc et al. 1993; Manger et al. 2002). The features of MRSS neurons such as large bilateral receptive fields and multisensory properties clearly differentiate this region from the adjoining SI area, but they are very similar to features reported in other regions regarded as higher order somatosensory representations (Bennett et al. 1980; Clemo and Stein 1983; Cusick et al. 1989; Manger et al. 2002; Robinson and Burton 1980). Therefore, it seems appropriate to regard MRSS not only as a somatosensory area that is distinct from SI, but also as a higher order somatosensory representation.

Possible homologies

The somatosensory, auditory and multisensory response properties as well as the sulcal location of the MRSS correlate closely with that recently described for cat rostral suprasylvian sulcal cortices (Monteiro et al. 2003; Clemo et al. 2007). Furthermore, if general homologies with cat somatosensory cortices can be assumed, MRSS probably could not be regarded as either the second (SII) and fourth (SIV) somatosensory areas. In the cat, SII is located on the suprasylvian gyrus (Berman 1961a, b; Carreras and Andersson 1963; Haight 1971; Woolsey and Fairman 1946) directly anterior to the AAF, and area SIV is located lateral/ventral to it in the adjoining sulcal bank (Clemon and Stein 1983). These locations of SII and SIV would most likely correspond, in ferret, to the anterior ectosylvian gyrus, anterior to the AVF/ADF auditory fields, and the dorsal bank of the pseudosylvian sulcus, respectively. While somatosensory projections have been identified to this area of the pseudosylvian sulcus in ferrets (Ramsay and Meredith 2004), their organization has not been studied.

Multisensory properties

The multisensory properties of the MRSS described here contribute to the growing literature giving shape to basic principles of multisensory organization and processing in cortex. Similar to other cortical studies of multisensory processing (Allman and Meredith 2007; Allman et al. 2008a, b; Dehner et al. 2004; Meredith et al. 2006), bimodal neurons (responsive to both somatosensory and auditory stimuli) in MRSS showed surprisingly low levels of integration when combined-modality stimuli were presented. Although it cannot be ruled out that these effects might be due to a ceiling effect (whereby unimodal stimuli drive neuronal responses to near maximum levels), these response levels may also be due to functional modes that establish restricted ranges of activation (as established for multisensory neurons in the superior colliculus; Perrault et al. 2005). In support of this notion, bimodal neurons that were facilitated when presented combined-modality stimuli had higher modality-specific (e.g., somatosensory

alone) firing rates than those that showed suppression, and all bimodal neurons had higher average firing rates over all stimulus conditions than their unimodal counterparts.

The present finding that some unimodal neurons in the MRSS revealed multisensory influences when tested with combined somatosensory and auditory stimuli is similar to other studies of cortical multisensory processing (Allman and Meredith 2007; Allman et al. 2008b; Carriere et al. 2007; Dehner et al. 2004; Driver and Noesselt 2008; Meredith et al. 2006). These subthreshold multisensory neurons appeared to be responsive to only one sensory modality when stimuli were presented separately, but revealed significant response modulations when that effective stimulus was paired with one from another apparently ineffective modality. Subthreshold multisensory neurons have been suggested not only to extend the range over which multisensory convergence occurs, but also to provide an intermediary level of response integration between that produced by bimodal neurons and the lack of integration in neighboring unimodal neurons (Meredith and Allman 2009).

It is also surprising that, despite the presence of a population of multisensory neurons (bimodal as well as subthreshold), the response of this cortical area as a whole appeared unaffected by the presence of a multisensory stimulus. Although analysis methods other than spike counts (e.g., mutual information) might detect multisensory effects, the lack of a demonstrable multisensory effect in the MRSS population is undoubtedly due to the consistently low levels of integration generated in the multisensory neurons of this region.

Multisensory transition zones

Numerous studies have identified areas where bimodal neurons respond to inputs from the different sensory modalities represented in adjoining cortical regions (Allman and Meredith 2007; Allman et al. 2008b; Brett-Green et al. 2003; Hunt et al. 2006; Meredith 2004; Wallace et al. 2004). Consistent with these observations, multisensory (somatosensory–auditory) neurons were identified in the MRSS (and also in the adjacent sulcal area of the lateral rostral suprasylvian sulcal cortex; Keniston et al. 2008), regions located between somatosensory and auditory cortices in the ferret. However, comparison of these different studies indicates that the organization of multisensory transition zones can vary widely. For example, in the cat, the multisensory transition between auditory area DZ and visual PLLS is restricted to a narrow band (<1 mm thick) of bimodal neurons that were largely *exclusive* of the wider distribution of subthreshold multisensory neurons (Allman and Meredith 2007; Clemo et al. 2008b). In contrast, in the transition between auditory FAES and visual AEV in cats, the distribution of bimodal and subthreshold multisensory neurons seems to be intermingled (Meredith and Allman 2009; Wallace et al. 2004) as they were in ferret MRSS. However, the functional significance of these differences in distribution of multisensory neuron types in different cortices remains to be determined.

Conclusions

The MRSS of ferret cortex was found to be primarily responsive to low-intensity cutaneous stimulation, with regions of the head, neck and upper trunk represented somatotopically. Anatomical connections arose largely from the corresponding head representation of SI. However, unlike SI, the MRSS exhibited a different cytoarchitecture, bilateral somatosensory receptive fields, and auditory responses/influences. Multisensory neurons in MRSS represented 29% of the sample and included both bimodal and subthreshold multisensory types. These observations indicate that MRSS is a separate somatosensory representation from SI that demonstrates features of higher level somatosensory cortex as well as transitional properties of a region located between somatosensory and auditory areas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Location of the medial bank of the rostral suprasylvian sulcus (MRSS). The suprasylvian sulcus (*opened and shaded gray*) is located on the lateral surface of the ferret cortex. The MRSS is bordered by SI cortex (medially), and auditory cortices (laterally) and extends posteriorly to the level of the caudal most extent of the pseudosylvian sulcus (*gray arrow*). Visual areas AMLS and ALLS are located caudally. The anterior sulcal region has not been studied. The location of the MRSS is also indicated by the *gray shaded areas* on the coronal sections. *A1* primary auditory region, *AAF* anterior auditory field, *ADF* anterior dorsal field, *AVF* anterior ventral field, *LRSS* lateral bank of the rostral suprasylvian sulcus, *PPr* posterior parietal rostral, *SI* primary somatosensory area, *SII* secondary somatosensory area, *SIII* third somatosensory area

Fig. 2.

Cytoarchitecture of MRSS. The cytoarchitecture of the MRSS was examined in tissue prepared with the antibody SMI-32. The micrograph (*left*) shows the suprasylvian gyrus, sulcus (*arrow*) and medial bank (MRSS). On the gyrus (corresponding to SI), layers II–III are relatively compact and layer IV is wide. In contrast, within MRSS layers II–III become expanded while layer IV is diminished. These features are compared in camera lucida drawings (*right*) of the areas of SI and MRSS (indicated by the *boxes*). *Roman numerals* indicate cortical lamina; *scale bar* 250 μ m

Fig. 3.

Electrophysiological responses of different neuron types in the MRSS. Responses of a **a** somatosensory neuron, **b** auditory neuron, **c** bimodal neuron are shown. In **a**, the auditory stimulus had no effect on the somatosensory responses of the neuron, whether delivered alone or in combination. Similarly, in **b**, a somatosensory stimulus did not affect the responses of an auditory neuron. The bimodal neuron in **c** responded to auditory and somatosensory stimuli delivered alone, and combined sensory stimulation generated only a modest response increase. Raster: 1 dot = 1 spike; 50 trials. Histogram: 10 ms time bins. Stimulus traces: A auditory, S somatosensory

Fig. 4.

Distribution and location of sensory neurons in the MRSS. In **a**, a coronal section indicates the region of the MRSS (*gray*), which is magnified in **b–f**. Part **b** illustrates the location of all electrode penetrations ($n = 14$; animals = 5) made into the MRSS. Among these penetrations, the majority of neurons responded only to somatosensory stimulation and these were found throughout the MRSS (**c**; *gray dots*). A few neurons were responsive only to auditory stimulation, and were located in the supragranular layers (**d**; *gray dots*). Bimodal cells responsive to both auditory and somatosensory stimulation were scattered throughout the MRSS (**e**; *black dots*). Subthreshold multisensory neurons (unimodal somatosensory neurons

significantly influenced by the presence of auditory stimuli) were also scattered throughout the MRSS (f; *black dots*)

Fig. 5.

Organization of tactile receptive fields in the MRSS. The lateral view of the brain (*right*) indicates the A-P level from which the coronal sections (**a–e**) were taken. On each section, *the line* through the MRSS indicates the electrode penetrations that passed from lip-to-fundus of the sulcal bank. *The dashed lines* along each penetration bracket the region where the tactile neurons with receptive fields (depicted below) were recorded. In the bottom panel, tactile receptive fields, which were mapped at 200 μm intervals along each penetration, are arranged vertically beneath the corresponding cortical section. Recording sites at corresponding depths in different electrode penetrations are aligned horizontally. It can be seen that the tactile receptive fields shift from relatively small portions of the (rostral) face (**a, b**) to larger receptive fields on the face or head (**c, d**), and finally onto the caudal head and neck/upper torso (**e**) as recording penetrations progressed from anterior to posterior. Similarly, a progression from small to large receptive fields was found from superficial to deep along the penetrations. In addition, there was a high proportion of bilateral receptive fields (indicated by *black dots*),

particularly in the caudal most sections. Neurons that responded to both tactile and auditory stimulation are denoted by 'A'

Fig. 6. Tactile receptive fields of SI neurons. On each coronal section through SI (A anterior, D posterior), the numbered recording sites correspond to the dots on the lateral view of the brain, as well as the tactile receptive fields illustrated below. Most of the SI tactile receptive fields were restricted to small, contralateral regions of the cheek, nose or vibrissa region. Only one bilateral receptive was found (indicated by black dot); none were bimodal

Fig. 7.

MRSS connections with SI. MRSS has a reciprocal projection to the head region of SI. In **a**, the location of labeled cells (*dots*) are plotted on serial sections **a–f** through SI (section location depicted on the lateral view of the brain). A micrograph of a labeled neuron is shown (*far right*; scale = 50 μm). In **b**, labeled boutons in the MRSS following an injection onto SI are shown on serial sections **a–e** (section locations correspond to those indicated on the lateral view of the brain). Labeled boutons are illustrated in the micrograph (*lower right*; scale = 10 μm). Note that labeled boutons were found across all laminae and that they became increasingly sparse at caudal sections and were absent in the most caudal section depicted. Injection sites depicted as *gray-filled areas*

Fig. 8.

Multisensory processing in MRSS neurons. *Scatter plots* show the neuronal activity elicited by somatosensory stimulation (*x*-axis) plotted against the change in response evoked by combined auditory–somatosensory stimulation (*y*-axis) for **a** all MRSS sensory neurons, and sub-types **b** bimodal, **c** subthreshold multisensory and **d** unisensory neurons. Identical response levels for these two measures fall on the line of unity (*x*-axis). Note that for each MRSS neuron sub-type, many responses showed little response change. In addition, bimodal neurons showed substantially higher absolute response levels (on both sensory tests) than did subthreshold or unisensory neurons. *Trend lines* are indicated on each graph (slope values: **a** 0.06, **b** 0.09, **c** −0.016, **d** 0.03). The average activity levels of MRSS neurons are illustrated in the *bar graphs*, showing the mean spikes/trial (with *standard error bars*) for the somatosensory (S, gray bars) and combined auditory–somatosensory (AS, black bars) stimulus conditions for each neuron type. Within the bimodal group, responses were significantly greater to the combined stimuli than to the somatosensory stimulus. Bimodal neurons also had significantly greater responses in both stimulus conditions (S and AS) than the subthreshold and unisensory types. Within the subthreshold multisensory group, responses were significantly lower to the combined stimuli than to the somatosensory stimulus. However, there was no significant difference in activity levels between the subthreshold multisensory and unisensory groups in response to the somatosensory stimulus. For the entire MRSS sample (**a** all neuron types), the average mean spikes/trial was not significantly different between somatosensory and combined somatosensory–auditory stimulus conditions (*far right bar graphs*). Average spontaneous activity levels are indicated by a *white line*. NS not significant; **p* < 0.05 paired *t* test

Table 1

The proportion of the total number of sensory neurons encountered in the MRSS that were unimodal and bimodal, as well as the different types of unimodal neurons

	Number (%) of sensory neurons
Unimodal	304 (84)
Somatosensory	251 (69)
Auditory	7 (2)
Subthreshold MS	46 (13)
Bimodal	59 (16)
Total sensory	363 (100)