Two Distinct Regions of Secondary Somatosensory Cortex in the Rat: Topographical Organization and Multisensory Responses

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Brett-Green, Barbara, Marcy Paulsen, Richard J. Staba, Eva Fifková, and Daniel S. Barth. Two distinct regions of secondary somatosensory cortex in the rat: topographical organization and multisensory responses. J Neurophysiol 91: 1327–1336, 2004. First published October 29, 2003; 10.1152/jn.00905.2003. In rodents, as in other species, regions of secondary somatosensory cortex (SII) may be distinguished from primary cortex (SI) both anatomically and electrophysiologically. However, the number of rodent SII subregions, their somatotopic organization, and their function are poorly understood. The presence of multisensory responsive neurons in some areas of SII suggests that one of its roles may be in the integration of somatosensory information with information from other sensory modalities. In this study, we used auditory, somatosensory, or combined auditory/somatosensory stimuli, and high-resolution epipial-evoked potential maps of rat SII to identify the number of spatially discrete subregions, estimate their somatotopic organization, and delineate regions with multisensory response properties. Maps revealed two distinct subregions within SII, one rostral and the other caudal, which were situated lateral to the posteromedial barrel subfield. Distinct somatotopeties were evident at both SII loci, and analysis of evoked responses within both areas indicated multisensory interactions. These data are consistent with the presence of classically defined rostral SII regions and provide functional evidence for a lesser known, but distinct, caudal SII area. Furthermore, evidence for multisensory interactions within SII suggests that both secondary areas may process features specifically associated with multisensory integration in parallel with unimodal processing in primary areas.

INTRODUCTION

Somatosensory cortex in the rodent and in other species is typically divided into primary regions that receive dominant projections from the specific relay nuclei of the thalamus and produce the shortest latency responses to afferent stimulation and secondary regions that are reciprocally connected with primary cortex and respond at longer latencies to afferent input. The somatotopic organization of a single primary somatosensory region (SI) has been well established in previous anatomical and electrophysiological studies of the rat (Chapin and Lin 1984; Hall and Lindholm 1974; Koralek et al. 1990; Wallace 1987; Welker 1971, 1976; Woolsey and LeMessurier 1948), mouse (Angel and Lemon 1975; Wallace 1987; Woolsey 1967), and squirrel (Krubitzer et al. 1986). However, the number and somatotopic organization of secondary somatosensory regions (SII) is still the subject of some debate.

A single area of SII in the rat (Akers and Killackey 1978; Chapin and Lin 1984; Fabri and Burton 1991; Koralek et al. 1990; Wallace 1987; Welker and Sinha 1972; Woolsey and LeMessurier 1948) and mouse (Carvell and Simons 1986, 1987; Wallace 1987; Woolsey 1967) has been identified lateral to SI, just rostral to auditory cortex. Somatotopic maps using evoked potential (Woolsey 1952; Woolsey and LeMessurier 1948) and microelectrode unit recordings (Welker and Sinha 1972) suggest an image of the rat’s body in this region that is upside down and oriented rostrally. However, more recent examination of ipsilateral cortical connections of SI (Fabri and Burton 1991) indicate somatotopically organized reciprocal connections between SI and two mirror image areas of secondary cortex in the lateral region: one overlapping classically defined SII with the same somatotopic organization as previously reported and a second inverted image located more laterally near the rhinal fissure. Further complicating the issue of the number of secondary somatosensory areas in the rat are tracing studies that suggest that, in addition to a lateral SII, there may also be a second separate area of SII reciprocally connected with SI but positioned in a more caudal and medial locus (Fabri and Burton 1991; Koralek et al. 1990), similar to separate rostral and caudal secondary somatosensory zones identified in the squirrel (Krubitzer et al. 1986).

Even less well understood is the function of SII compared with SI. Recent studies in the rodent, combining somatosensory with either auditory (Brett-Green et al. 2003; Carvell and Simons 1986, 1987; Di et al. 1994; Ramachandran et al. 1993; Wallace et al. 2004) or visual stimulation (Barth et al. 1995; Ramachandran et al. 1993; Wallace et al. 2004), have suggested that one of the functions of SII, and indeed of secondary sensory cortex in general, may be in multisensory integration. However, while cells within SII responding to multisensory stimuli have been reported, the relative topographies of multisensory zones and SII have not been investigated in detail.

In this study, we used high-resolution epipial-evoked potential mapping to determine if rat SII is composed of one or more spatially discrete regions and to estimate its somatotopic organization. Although field potential mapping has a lower spatial resolution than microelectrode recording, it has the advantage that wide areas of cortex can be recorded simultaneously and it eliminates spatial sampling errors inherent in single microelectrode recording. In addition, field potential maps can identify and separate distinct cortical regions based on spatio-temporal differences in the evoked response complex (Barth and Di 1991; Barth et al. 1993; Di and Barth 1991). We then compared maps of SII to regions where somatosensory and auditory...
responses overlap and interact to examine the involvement of secondary cortex in multisensory processing.

**METHODS**

**Animals and surgery**

All procedures were performed in accordance with University of Colorado Institutional Animal Care and Use Committee guidelines for the humane use of laboratory animals in biological research. Ten adult male Sprague-Dawley rats (300–400 g) were anesthetized to surgical levels using intramuscular injections of ketamine (71 mg/kg body weight), xylazine (14 mg/kg), and acepromazine (2.4 mg/kg), placed on a regulated heating pad, and maintained with subsequent injections throughout the experiment so that the eye blink reflex could be barely elicited. A unilateral craniectomy was performed over the right hemisphere, extending from bregma to lambda and from the mid sagittal sinus lateral to the temporal bone, exposing a wide region of parieto-temporal cortex where the dura was reflected. Animals were killed by anesthesia overdose without regaining consciousness at the conclusion of the experiment.

**Stimulation**

Auditory click stimuli were delivered with a high-frequency piezo-electric speaker aligned with, and approximately 10 cm from, the contralateral ear. The ipsilateral ear was blocked with soft wax. Clicks were computer-controlled monophasic square-wave pulses (0.3 ms; \( \sim 50 \text{ dB SPL} \) at 10 cm). Silent stimulation of individual vibrissae on the contralateral mystacial pad was achieved with a pulsed (0.5 ms) electromagnetic. Vibrissae were attached to a short ferrous wire about 10 mm from the mystacial pad. The wire was positioned 1.0 mm below the magnet. and pulsed (1 ms) stimulation displaced the vibrissae vertically by \( \sim 0.5 \text{ mm} \). Silent electrical stimulation of discrete body regions was achieved with a bipolar stainless steel electrode (0.5 mm separation; 1-mm exposed tip) attached to a constant current source, delivering current pulses (1 ms) of minimum current (0.2–0.5 mA) required to produce a reliable evoked response with no noticeable muscle contractions when applied to the shaved skin pretreated with conductive jelly.

**Data collection and analysis**

Epipial maps of auditory and somatosensory evoked potentials (AEPs and SEPs, respectively) were recorded using a flat multielectrode array consisting of 64 silver wires in an \( 8 \times 8 \text{ grid} \) (tip diameter: \( \sim 100 \text{ \mu m} \); inter-electrode spacing: 500 \( \mu \text{m} \)) covering a \( 3.5 \times 3.5 \text{ mm} \) area. Recordings were referred to a silver ball electrode secured over the contralateral frontal bone and were simultaneously amplified (10,000 times), analog filtered (band-pass cut-off = \( -6 \text{ dB at } 0.001–3,000 \text{ Hz} \), roll-off = 5 dB/decade), and digitized at 10 kHz. Trials of separately evoked AEPs or SEPs consisted of a 25-ms baseline followed by 75 ms of poststimulus activity, averaged over 50–100 presentations. Evoked responses in Figs. 1–4 reflect grand averages across six animals. Trials to examine multisensory interaction consisted of an initial auditory stimulus followed at 50 ms by a somatosensory stimulus. While simultaneous presentation of auditory and somatosensory stimuli were used to test multisensory interaction in our previous studies (Barth et al. 1995; Brett-Green et al. 2003; Di et al. 1994), the small amplitude SEPs evoked in SII by electrical stimulation of the skin in this study led us to use asynchronous presentation, because this was found to produce the strongest and most reliable interaction effects due to depression affected by inhibition following the response to the first excitatory stimulus. Multisensory potentials were averaged over 50–100 auditory, somatosensory, and auditory/somatosensory stimulations, randomized in their presentation sequence with an interstimulus interval of 1 s. Evoked responses in Figs. 5–7 reflect grand averages across the additional four animals used in the multisensory phase of the experiment. Single vibrissa-evoked SEPs were used to consistently align the array across animals, which, in previous studies, has been found to have a placement error of less than 0.5 mm when verified with subsequent histology. Latencies of evoked potential amplitude peaks are reported as mean \( \pm \text{ SEM milliseconds poststimulus} \) and, comparisons of latencies were made using paired \( t \)-test with significance set to \( P \leq 0.05 \).

**Cytochrome oxidase histochemistry**

A cytochrome oxidase (CO)-stained section through layer IV of the flattened right hemisphere of one animal was obtained to reveal the location and spatial extent of granular (dark) and dysgranular (light) regions to be provide a template for subsequent illustration of array placements in relation to these regions. The animal was perfused intracardially with 0.1 M phosphate buffer followed by modified peridate-lysine-parafomaldehyde fixative (pH 7.4). Before the removal of the brain, a sucrose buffer solution was rinsed through the animal. To obtain a complete section of layer IV, the naturally curved cortex was flattened (Welker and Woolsey 1974) and immersed in a 30% sucrose buffer solution and kept at 7°C for 48 h. Sections were cut tangential to the pia with a cryotome at a thickness of 40 \( \mu \text{m} \). Sections were incubated at 37°C in the dark for 1 h. The incubation medium was prepared immediately before use with 50 mg dimethyl sulfoxide and 5 g sucrose dissolved in 100 ml 0.1 M phosphate buffer. Before the introduction of the tissue sections, 95% O\(_2\)-5% CO\(_2\) was also bubbled in the solution for 1 min. Incubation was terminated when differentiation between highly reactive and nonreactive areas could be discerned. Sections were rinsed in three changes of 0.1 M phosphate buffer, mounted, air dried, and coverslipped.

**RESULTS**

A CO-stained section through layer IV of the flattened right hemisphere (Fig. 1A) revealed the location and spatial extent of granular (dark) and dysgranular (light) regions, permitting the identification of auditory cortex the postero medial barrel sub-field reflecting the vibrissa representation of SII (Fig. 1A; AUD and PMBSF, respectively) and spinal representations of S1 medial to the PMBSF. This section was used as a template for subsequent figures but should be considered approximate since no further histology was performed. A square \( 8 \times 8 \text{ electrode array recorded from an area of } 3.5 \text{ mm}^2 \) in a single placement (Fig. 1A; white square). Stimulation of a single vibrissa on the contralateral mystacial pad (Fig. 1B; C2; 5 rows of barrels corresponding to the major vibrissae are labeled A–E and 5 columns \( 1–5 \)) resulted in a positive/negative slow wave in the SEPs that was of earliest poststimulus latency and largest amplitude at electrode sites above the principal barrel corresponding to the C2 vibrissa (Fig. 1, B and C, green trace) but spread across an approximately 2-mm\(^2\) area, including cortex lying caudal to the PMBSF (Fig. 1, B and C, red trace). Enlargements of SEPs from these two locations indicated an approximately 7-ms delay in the poststimulus latency of the first positive amplitude peak of the posterior recording compared with the first positive amplitude peak at the C2 barrel, which has been determined in previous studies to be the hallmark of responses in secondary somatosensory (Barth et al. 1993) and auditory (Barth and Di 1991; Barth et al. 1993; Di and Barth 1992) cortex and was used to identify these regions throughout the remaining study. Normalized and interpolated (bi-cubic spline) maps of the initial positive amplitude peak of the SEP beginning at the earliest latency (Fig. 1D; 19 ms) and
progressing in 1-ms steps to the longest latency (Fig. 1D; 25 ms) indicated the separate loci of primary cortex in the PMBSF (Fig. 1D; C2) and secondary cortex (Fig. 1D; c2) activated by stimulation of the contralateral C2 vibrissa. Because of its precisely localized response and reproducibility across animals, short-latency SEPs to C2 stimulation were used to functionally align the surface array in all subsequent recordings.

At the medial array location depicted in Fig. 1, maps of the short latency primary SEP response that were averaged across animals (Fig. 2B; C2; 18.4 ± 1.4 ms) corresponded closely with the loci of peak SEP amplitudes for the individual animals (Fig. 2B; circles), centered on the C2 barrel of the PMBSF. Peak SEP amplitudes for the secondary C2 response (Fig. 2C; c2; 24.8 ± 2.6 ms) showed a similarly tight grouping. Subsequent maps of the primary short-latency SEP response to stimulation of the contralateral pinna (Fig. 2D; PN; 22.3 ± 0.7 ms), midtrunk (Fig. 2E; MT; 25.2 ± 0.6 ms), hindlimb (Fig. 2F; HL; 27.8 ± 1.2 ms), hindpaw (Fig. 2G; HP; 28.4 ± 1.3 ms), forelimb (Fig. 2H; FL; 23.5 ± 0.8 ms), and forepaw (Fig. 2I; FP; 23.1 ± 1.2 ms) indicated loci at more medial locations outside of the PMBSF and within the somatic representation of SI. A composite view of these responses (Fig. 2A) indicated an inverted somatotopic organization of primary cortex, with C2 (50% amplitude solid isocountour line and lettering color coded in black), PN (red), MT (orange), and HL (dark green) aligned along the lateral to medial axis, and the FL (dark blue), FP (light blue), and HP (light green) positioned rostrally. In this and subsequent illustrations, uppercase letters with solid contour lines were used to denote primary sensory zones, and lowercase letters with dashed contour lines labeled secondary cortex. Color codes for the various stimulated body parts remain consistent unless otherwise indicated.

At a more lateral position of the recording array, SEPs averaged across animals revealed loci of SII (Fig. 3). Stimulation of the C2 vibrissa produced a delayed response approximately 2 mm lateral to the border of the PMBSF, suggesting a distinct and complementary area of SII (Fig. 3C) that was rostral and lateral to the region identified in Fig. 2. Similarly, secondary responses to fl (Fig. 3D), hl (Fig. 3F), and mt (Fig. 3H) revealed two separate islands located at rostralateral and caudomedial regions of the array. At this location, secondary responses to both fp (Fig. 3E) and hp (Fig. 3G) could only be detected at the rostralateral locus, and those associated with pn (Fig. 3I) were only detected in the caudomedial locus. For the purpose of anatomical reference, the earliest latency response of the AEPs is also shown (Fig. 3B) and was centered on
primary auditory cortex. At the rostrolateral locus, poststimulus latencies of the secondary responses were significantly delayed compared with corresponding response latencies in primary cortex (delay in ms: \(c_2 = 5.9 \pm 2.1, P < 0.01; \ fl = 3.8 \pm 1.1, P < 0.01; \ fp = 4.1 \pm 1.9, P < 0.05; \ hl = 4.6 \pm 1.5, P < 0.01; \ hp = 5.2 \pm 2.5, P < 0.05; \ mt = 8.0 \pm 3.5, P < 0.01\)). Poststimulus latencies of secondary responses at the caudomedial locus were also significantly delayed compared with corresponding primary responses (delay in ms: \(fl = 3.6 \pm 1.0, P < 0.01; \ hl = 4.8 \pm 1.6, P < 0.01; \ mt = 7.5 \pm 3.2, P < 0.01\); \(pn = 5.0 \pm 1.6, P < 0.01\)), but in no cases were there significant differences between the rostrolateral and caudomedial response latencies where they co-occurred (i.e., \(fl, hl,\) and \(mt\)). A composite diagram (Fig. 3A) indicated a great deal of overlap within the two regions of SII, obscuring their somatotopic organization. This was exacerbated by poor coverage of the array over the caudomedial region. However, the rostrolateral region revealed a rough somatotopic arrangement, with \(fl\) and \(fp\) positioned rostral to \(hl, hp,\) and \(mt\).

**FIG. 2.** Somatotopic mapping of the primary SEP response, averaged across animals, to stimulation of various body parts. A: composite of 50% amplitude isocontour lines from normalized SEP responses reflect approximate primary representations of the C2 vibrissa/barrel (black), Pinna (PN; red), midtrunk (MT; orange), hindlimb (HL; dark green), hindpaw (HP; light green), forelimb (FL; dark blue), and forepaw (FP; light green). Response pattern is somatotopically organized, with the head oriented laterally and the limbs rostrally. A secondary response to vibrissa stimulation (c2) is also depicted with a black dashed line. B–I: interpolated maps of the grand average SEP with superimposed peak amplitude responses for individual animals (circles) are shown for C2, c2, PN, MT, HL, HP, FL, and FP, respectively.

**FIG. 3.** Somatotopic mapping of the grand averaged secondary SEP response at a more lateral array location indicates distinct rostral and caudal zones. A: responses to stimulation of different body parts are color coded in the same way as Fig. 2. However, dashed isocontour lines and lower case lettering are used to label secondary as opposed to primary responses. Secondary response to vibrissa stimulation (c2) is lateral to the PMBSF and indicates a complementary and distinct area of secondary cortex. Similarly, stimulation of other body parts yielded secondary responses in 2 distinct regions, the 1st situated lateral to and the 2nd caudal to the PMBSF. While responses in both of these regions suggested a somatotopic organization within each, the degree of overlap precludes more precise delineation. Short latency responses of the auditory evoked potential (AEP) are also depicted over primary auditory cortex (AUD; purple) for reference. B–I: grand averaged maps with superimposed peak amplitude responses for individual animals are shown for separate stimulation conditions.
A more posterior positioning of the electrode array provided better coverage of posterior SII (Fig. 4). Here, both the rostral-lateral and caudomedial areas of secondary C2 cortex may be seen to be concurrently activated (Fig. 4C). Posterior secondary regions of fl (Fig. 4D), fp (Fig. 4E), hl (Fig. 4F), hp (Fig. 4G), mt (Fig. 4H), and pn (Fig. 4I) were more clearly delineated. Again, secondary responses were significantly delayed compared with corresponding primary responses but did not significantly differ in latency between the caudomedial and rostrolateral locus when both were activated. In addition, both the primary auditory response (Fig. 4B; AUD) and secondary response (Fig. 4B; aud) could be discerned and were in close agreement with previously published reports (Barth and Di 1990, 1991; Brett et al. 1994; Di and Barth 1992, 1993). Despite the remaining spatial overlap in the posterior SII responses, an approximate somatotopic organization was suggested in the composite (Fig. 4A), with rostral body parts positioned rostral to the caudal body parts.

During the multisensory interaction paradigm, auditory stimuli were presented at the beginning of the 100-ms recording epoch and somatosensory stimuli at a 50-ms delay. Auditory and somatosensory stimuli were presented either separately or in tandem to test for the effect of auditory stimuli on the subsequently evoked ASEP (auditory/somatosensory evoked potential). Figure 5A depicts a grand average across animals of the SEP response in SII to forepaw stimulation alone (Fig. 5A; black traces) with maximum amplitude in the rostral and extreme lateral region of the array. When auditory stimuli were presented alone, the grand average AEP (Fig. 5B; red traces) covered the primary auditory cortex but also spread rostrally (Fig. 5A; dashed red contour line = 25% peak amplitude of AEP), partially overlapping the separately evoked SEP response (Fig. 5A; dashed black contour line = 25% peak amplitude of SEP). A model (Fig. 5D; blue), computed as the sum of the separately evoked SEP and AEP, closely resembled the SEP alone (Fig. 5B) since the AEP complex at this latency was attenuated (Fig. 5C). However, when somatosensory stimuli were preceded by auditory stimuli in the same trials, multisensory interaction was evident. A preceding auditory stimulus both attenuated the subsequently evoked ASEP and increased its poststimulus latency (Fig. 5D; green trace) compared with the sum of the separately evoked responses. This deviation in ASEP response pattern from a sum of the AEP and SEP indicates neuronal interaction between the auditory and somatosensory stimulus, since, without interaction, the volume currents would simply sum linearly. Multisensory interaction was quantified at each electrode site by subtracting the summed unimodal responses (MODEL) from the response to combined stimulation (AUD/SOM), yielding a difference waveform (Fig. 5E; DIFF). In the example in Fig. 5, a normalized map of the maximum amplitude of this difference waveform (Fig. 5F) indicated that multisensory interaction included the forepaw representation of SII, but was more widespread, including caudal regions where the unimodal AEP and SEP spatially overlapped.

Maps of multisensory interaction, computed in this way for stimulation of other body regions (Figs. 6 and 7), displayed a similar pattern. Interaction was apparent in responses recorded from both the rostrolateral and caudomedial regions of SII and for every body region stimulated. Loci of multisensory interaction generally followed the pattern established for separately evoked SII responses. However, these regions were more widespread than corresponding secondary SEP responses. In addition, loci of multisensory interaction were shifted toward auditory cortex compared with the corresponding loci of maximum unisensory responses in SII. This is particularly apparent in composite maps of Fig. 8, where direct comparisons can be made between the somatotopic regions of SII (Fig. 8A) and multisensory interaction zones (Fig. 8B). While the somatotopic organization of multisensory foci in the caudal area of SII
was still discernible, that in the rostral area was distorted, primarily by a shift of the forepaw and forelimb representations from their rostral location to more caudal and medial loci that were closer to auditory cortex.

**Discussion**

These results, obtained with epipial-evoked potential mapping, reveal the somatotopic organization of SI and two distinct regions of SII that are positioned lateral to the PMBSF at rostral and caudal loci and are separated by approximately 2 mm (termed SIIrost and SIIcaud, respectively). Both SIIrost and SIIcaud display multisensory interaction. The regions of multisensory interaction substantially overlap secondary somatosensory loci but are shifted toward auditory cortex.

The topography of SI, derived here from SEP maps, agrees well with previous anatomical and electrophysiological studies in the rat (Chapin and Lin 1984; Hall and Lindholm 1974; Koralek et al. 1990; Wallace 1987; Welker 1971, 1976; Woolsey and LeMessurier 1948), mouse (Angel and Lemon 1975; Wallace 1987; Woolsey 1967), and squirrel (Krubitzer et al. 1986), indicating an inverted ratunculus. Early latency and large amplitude components of the primary SEP response are accompanied by longer latency and smaller amplitude responses clustered in two distinct islands positioned 2–3 mm lateral to the primary response, suggesting two separate areas of SII. The most rostral of these areas (SIIrost) corresponds to classically defined SII in the rat (Akers and Killackey 1978; Chapin and Lin 1984; Fabri and Burton 1991; Koralek et al. 1990; Wallace 1987; Welker and Sinha 1972; Woolsey and LeMessurier 1948) and mouse (Carvell and Simons 1986, 1987; Wallace 1987; Woolsey 1967). Spatial overlap of evoked responses in this region makes detailed determination of its somatotopic organization difficult. However, at least on the rostrocaudal axis, a topographic organization is evident, with the forelimb and forepaw positioned rostral to the hindlimb and hindpaw representations. Positioning of the hindlimb, midtrunk, and forelimb lateral to the hindpaw representation (forepaw overlapped too much with forelimb to make a clear distinction) suggests an image of the rat’s body that is upside down, similar to previous somatotopographic maps using evoked potential (Woolsey 1952; Woolsey and LeMessurier 1948) and microelectrode unit recordings (Welker and Sinha 1972). Location of the secondary representation of the C2 vibrissa is consistent with more complete evoked potential mapping of the major vibrissae (Barth et al. 1993; Di et al. 1994), indicating a large vibrissa representation of SII just lateral to the PMBSF (Carvell and Simons 1986, 1987; Fabri and Burton 1991; Koralek et al. 1990). Curiously, we were unable to record secondary responses to pinna stimulation in SIIrost, despite the substantial responses detected in SIIcaud. This may reflect an incomplete somatotopic representation in SIIrost or inadequate sampling of the area due to the presence of the temporal bone. We are presently performing labeling studies of intracortical pathways between electrophysiologically identified areas of primary somatosensory cortex and SIIrost and SIIcaud that may help to clarify this issue.

![Image of electrode recordings and SEP maps](http://jn.physiology.org/)

**FIG. 5.** Procedure for identifying regions of auditory/somatosensory multisensory interaction from epipial-evoked potential maps. A: averages across animals (n = 4) of separately computed AEP (red) and forepaw-evoked SEPs (black) are superimposed. Note that SEP is consistently later than AEP due to delayed presentation of somatosensory stimuli described in METHODS. The 25% peak amplitude isoncontour lines of AEP and SEP (red and black dashed traces) reveal a region of overlap (circled trace) between the unimodal response distributions. A 25% instead of 50% isoncontour line was chosen to better reflect the spatial distribution of small amplitude AEP and SEP since these have the widest spatial distribution and thus reveal the greatest overlap. B: enlargement of 2nd half of trace circled in A, depicting SEP at this electrode site. C: similar enlargement of AEP from the same location shows no residual response at this latency. D: model consisting of sum of separately evoked AEP and SEP (MODEL; blue) presents a waveform that is both earlier in latency and larger in amplitude than the actual response at this location when both stimuli were presented in tandem (AUD/SOM; green), indicating that ASE is attenuated and delayed when evoked following an auditory primary stimulus. E: nonlinear multisensory interaction was quantified by computing a difference waveform (DIFF) between multisensory response and linear model of summed unisensory responses. F: spatial distribution of multisensory interaction is visualized with a normalized interpolated map of peak amplitude of the difference waveform computed at each electrode site. Here, it may be seen that, while the largest amplitude secondary SEP response to forepaw stimulation was localized in the lateral and rostral region (A), the largest area of multisensory interaction shifted slightly caudally where unimodal AEP and SEP spatially overlapped.
There are several striking differences between the present responses from SIIrost recorded here and previous studies of SII. In a recent examination of ipsilateral cortical connections of SI, Fabri and Burton (1991) described somatotopically organized reciprocal connections between SI and two mirror image areas of secondary cortex, one just lateral to the PMBSF that they termed SII and a second inverted image located more laterally near the rhinal fissure termed the parietoventral (PV) area. We detected no distinct PV representation using evoked potential mapping. While this may attributed in part to the relatively poor spatial resolution of field potentials due to volume conduction, the trunk and proximal limb representations in their mirror image maps are separated by ≥1 mm, which should be resolvable with the present mapping technique. As noted earlier, we may have missed part of PV due to its extreme lateral location, beyond the borders of our craniectomy, which stopped at the temporal bone. However, it is interesting to note that their trunk representation in PV is similar to our midtrunk representation in SIIrost. Their trunk representation in SII is placed near the caudal border of the PMBSF, similar to other studies (Carvell and Simons 1986, 1987; Koralek et al. 1990; Woolsey 1967), and close to the midtrunk representation of SIIcaud in this study. Thus a possible explanation for discrepancies between the present and past descriptions of SII topography is that previous reports may have combined into one composite secondary somatosensory zone, parts of what we have defined as a clearly separate somatotopic map in the SIIcaud.

In all SEP maps of this study, both SIIrost and SIIcaud are evident, each with its own topography. Secondary-evoked responses in SIIcaud are often of equal or larger amplitude than in SIIrost, particularly for the pinna and midtrunk, but separate responses for each body region under examination reveal sep-

**FIG. 6.** Somatotopic organization of multisensory interactions produced by auditory stimuli followed by stimuli of various body parts. A: color codes for responses to stimulation of different body parts are the same for prior figures. Dashed circles represent the 50% isocontour lines of normalized difference waveforms within secondary somatosensory cortex. In most cases, the region of multisensory interaction corresponded approximately with similar regions of secondary SEP responses evoked during unisensory stimulation. However, these regions were more widely distributed, precluding detailed determination of somatotopic organization and demonstrated a tendency to shift from the locations of unisensory foci in the direction of the borders of auditory cortex, where unisensory AEP and SEP maximally overlapped. B–H: grand averaged maps of multisensory difference waveforms with superimposed peak amplitude responses for individual animals are shown for separate stimulation conditions.

**FIG. 7.** Similar to Fig. 6, but showing multisensory interactions at a more caudal array location, better covering the caudal secondary somatosensory region.
arate secondary loci in rostral and caudal zones. The location and somatotopic organization of SIIcaud in the rat is quite similar to “SII” defined for the squirrel by Krubitzer et al. (1986), with their separately identified PV corresponding in location and approximate somatotopic organization to the present SIIrost in the rat. Given the consistently large responses to somatosensory stimulation that are evident in SIIrost, it is puzzling why this region is rarely noted in studies of the rat. This may reflect in part a methodological advantage of high resolution evoked potential mapping, where widespread cortical regions can be simultaneously mapped and spatiotemporal differences in the SEP may be exploited to uniquely identify and separate activated regions. However, this area has not gone unnoticed. Small injections of fluorescent tracers, particularly into the trunk or distal limb zones of SI, label a distinct parietal lateral region (PL; Fabri and Burton 1991) that corresponds closely with our SIIcaud. The same injections concurrently label regions within the SIIrost, suggesting dual representations in these areas. In a similar study, injections of tracer into the SI hindpaw area, which also appear to have spread into portions of the forepaw and trunk areas, labeled cells in SIIrost, but less dense label was also found farther caudomedially where we have identified SIIcaud (Koralek et al. 1990). While these authors attributed the caudomedial label to diffusion of the tracer injection onto the SI trunk representation, in light of the present results, these data may have actually reflected separate representation of the limbs as well as the trunk in SIIcaud. In the present results, the forelimb, pinna, midtrunk, and hindpaw representation within SIIcaud is positioned at progressively more caudal locations, indicating a somatotopic organization oriented similar to that of SIIrost. Whether the secondary response to C2 vibrissa stimulation, positioned caudal to the PMBSF, represents a complementary vibrissa representation of SIIcaud cannot be stated with any certainty without more systematic vibrissa mapping, which was not performed here. An alternative explanation is that it may lie within anterior extrastriate cortex where neurons that respond to whisker deflection as well as visual stimuli have been detected in mice (Wagor et al. 1980) and may therefore belong to a separate body map.

SEPs from both SIIrost and SIIcaud spatially overlap click-evoked AEPs, which is evident in the rostral and medial belt areas of auditory cortex (Patterson 1977), suggesting that cells in these regions may have multisensory response properties (Barth et al. 1993, 1995; Brett-Green et al. 2003; Di et al. 1994). This possibility is confirmed by spatiotemporal interactions observed between the AEP and SEP when auditory and somatosensory stimuli are presented in tandem. Combined stimulation always produces a response that differs from the linear sum of separate auditory and somatosensory responses, with the greatest interaction evident near auditory cortex where the AEP and SEP maximally overlap (for a notable exception to this general rule, the multisensory c2 response is actually shifted slightly away from the c2 response to unimodal stimulation in SIIrost). Maps of these interaction patterns indicate that both SIIrost and SIIcaud have the capacity to participate in multisensory integration, a conclusion that is consistent with a recent report from our laboratory describing a multisensory zone (MZ) in rat cortex that extends along the border between primary auditory cortex and the PMBSF (Brett-Green et al. 2003). This general organizational pattern of multisensory cortex occupying the transitional areas between primary sensory cortices has also been noted in other recent microelectrode studies of the rat (Wallace et al. 2004).

While our previous work concentrated only on interactions between auditory- and vibrissa-evoked responses, the present results extend these findings to spinal representations of SII. The involvement of SIIrost in multisensory integration is consistent with earlier electrophysiological studies in the rodent (Carvell and Simons 1986, 1987; Koralek et al. 1990; Welker and Sinha 1972; Welker et al. 1988; Woolsey 1967), indicating that spinal representation within classically defined (rostral) SII is responsive to auditory stimulation. The present finding that SIIcaud also responds to auditory stimulation might have been anticipated from previous examination of thalamocortical input to this region (Brett-Green et al. 2003). Injection of small
quantities of retrograde tracer into a region overlapping what we now know as the pinna, midtrunk, forelimb, and forepaw region of SIIcaud, labels cells in the ventral posterior lateral (Vpl) and posterior (Po) nuclei of somatosensory thalamus as well as the ventral and medial divisions of the medial geniculate (MGv and MGm, respectively) and the suprageniculate (Sg) nuclei of acoustic thalamus. Thus while SIIcaud may receive sparse intracortical projections from SI (Fabri and Burton 1991; Koralek et al. 1990), it has access to both somatosensory and auditory afferent input via direct thalamocortical pathways. A similar picture may apply to SIIrost, which has well-defined intracortical projections from SI (Fabri and Burton 1991; Koralek et al. 1990). The loci of multisensory interactions in SIIrost, at the rostral border of primary auditory cortex, overlaps the secondary auditory belt cortex. SIIrost therefore probably receives direct auditory input from the acoustic thalamus (Arnault and Roger 1990; Brett et al. 1994; Patterson 1977; Ryugo and Killackey 1974; Winer and Larue 1987) as well as input from the somatosensory thalamus (Tracey and Waite 1995).

The function of SII in general, and consequently the differential function of SIIrost versus SIIcaud identified in this study, is largely unknown. Due to the wider and largely overlapping receptive fields in SII compared with SI, it may be plausible that SII serves as a higher stage in the processing of somatosensory information (Killackey 1983), serving to integrate information across broader body regions and possibly to integrate submodalities of somatosensation segregated in SI. However, evidence from this study indicates that another function of SII, and that of secondary cortex in other sensory modalities, is in the parallel processing of features concerned with multisensory integration. Ipsilateral connections between SI and SII are not unidirectional from primary to secondary, but reciprocal. It is apparent, at least in the rat, that SII receives as much if not more thalamic input as it does from SI (Brett-Green et al. 2003), suggesting a role in parallel processing as well as in hierarchical processing. While multisensory regions are shifted slightly toward the border of auditory cortex, they are partially co-localized with SIIrost and SIIcaud, respectively, suggesting that many but not all cells in these zones perform multisensory integration. It can only be speculated why there are two separate regions of SII in the rat and what their functional differences might be. It is interesting to note that SIIcaud identified here is adjacent to and partially overlaps a region of secondary visual cortex in the rat that responds to both visual and auditory stimuli (Barth et al. 1995), which was recently confirmed by Wallace et al. (2004). Thus in general, substantial subregions of the secondary sensory cortices might be sites of augmented responsiveness to a combination of sensory stimuli. In the specific case of SIIrost versus SIIcaud, the difference could be in the integration of bimodal (som/aud) versus trimodal (som/aud/vis) stimuli, respectively. The fact that, in the rat, there are two separate SII regions could be viewed as an expression of the overwhelming importance of somatosenses for this species.

GRANTS

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REFERENCES


