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MULTISENSORY PLASTICITY IN CONGENITALLY DEAF MICE: HOW ARE CORTICAL AREAS FUNCTIONALLY SPECIFIED?

D. L. HUNT,[a,b] E. N. YAMOAH[a,c] AND L. KRUBITZER[a,b]*

[a]*Center for Neuroscience, University of California, Davis, Center for Neuroscience, 1544 Newton Court, Davis, CA 95616, USA*

[b]*Department of Psychology, University of California, Davis, Center for Neuroscience, 1544 Newton Court, Davis, CA 95616, USA*

[c]*Department of Otolaryngology, University of California, Davis, Center for Neuroscience, 1544 Newton Court, Davis, CA 95616, USA*

Abstract—The neocortex of congenitally deaf mice was examined using electrophysiological recording techniques combined with cortical myeloarchitecture. Our results indicate that relative activity patterns across sensory systems during development contribute to modality assignment of cortical fields as well as the size of cortical fields. In congenitally deaf mice, "auditory cortex" contained neurons that responded to somatosensory, visual, or both somatosensory and visual stimulation; the primary visual area contained a larger proportion of neurons that responded to somatosensory stimulation than in normal animals, and the primary visual area had significantly increased in size. Thus, cortical architecture and functional specification were de-correlated. When results are considered in the light of molecular studies and studies in which peripheral activity is altered in development, it becomes clear that similar types of changes to the neocortex, such as alterations in cortical field size, can be achieved in more than one way in the developing and evolving neocortex.

Key words: deaf mouse, multisensory plasticity, evolution, cortical organization.

One of the most significant modifications made to the evolving human brain has been the remarkable expansion of the cortical sheet and an increase in the number of cortical fields that compose the neocortex. A cortical field is most often defined by its architectonic, functional, and connectional distinctions, and processing networks composed of these fundamental elements are believed to endow mammals with sophisticated sensory, perceptual, and cognitive abilities. Because of the important role that cortical fields play in generating these abilities, it is not surprising that there has been a heightened interest in both the genetic and environmental influences that shape cortical fields during development. As a result, a number of elegant studies have used a variety of genetic manipulations to determine the intrinsic molecular contributions to this developmental process. For example, recent studies have demonstrated that a particular gene and/or the combinatorial action of several genes sets up a number of features of cortical organization including relative geographic location of cortical fields (Bishop et al., 2000; Garel et al., 2003), the size of cortical fields (Hamasaki et al., 2004), the thalamocortical connections of fields (Bishop et al., 2000, 2003; Dufour et al., 2003), and aspects of corticocortical connections (Bishop et al., 2003;). It should be noted that some of these studies have demonstrated that the expression patterns of particular genes can be correlated with some cortical field locations, but a direct causal relationship between a particular gene, or combination of genes, and a functional cortical field and its connections in a normal system has yet to be established. Further, while manipulation studies in the form of knockout and transgenic mice, as well as studies utilizing electroporation techniques, have demonstrated possible genetic mechanisms that instruct cortical field organization, it is not known if the mechanisms uncovered with these techniques operate naturally in biological systems.

Thus, there are still a number of fundamental questions that have yet to be addressed regarding the mechanisms that give rise to particular aspects of cortical organization and how these mechanisms evolve. We have begun to consider two such questions. First, what determines the size of a sensory domain? We define a sensory domain as the extent to which a given sensory system occupies a particular amount of space on the cortical sheet. Second, what determines the size of a cortical field? We raise these questions because sensory domain assignment, functional organization, and the relative size of cortical fields are features that differ markedly across species. For example, mammals such as the duck-billed platypus have an enormous amount of cortex devoted to processing somatic and electrosensory inputs from the bill (Krubitzer et al., 1995), while in squirrels and opossums, the majority of cortex is devoted to processing inputs from the retina (Fig. 1). This type of unequal sensory domain assignment is observed in all species examined, to a greater or lesser extent (Johnson, 1990; Krubitzer and Kahn, 2003 for review). In addition to differences in sensory domain organization, the relative size of cortical fields also varies dramatically, even in closely related species. The size of the primary visual area, V1, the secondary visual area, V2, and the middle temporal visual area, MT (see Table 1 for abbreviations), relative to the size of the cortical sheet can differ markedly

*Correspondence to: L. Krubitzer, Center for Neuroscience, 1544 Newton Court, Davis, CA 95616, USA. Tel: +1-530-757-8868; fax: +1-530-757-8827.
E-mail address: lakrubitzer@ucdavis.edu (L. Krubitzer).

Abbreviations: AAF, auditory field; ABR, auditory brainstem response; A1, primary auditory area; A1+AAF, primary auditory area+anterior auditory field; CO, cytochrome oxidase; DPOAE, distortion product otoacoustic emission; NKCC1, Na^+-K^+-$2Cl^-$ cotransporter; PMCA2, Ca^{2+}-ATPase isoform 2; SPB, sucrose phosphate buffer; S1, primary somatosensory area; S2, secondary somatosensory area; V1, primary visual area; V2, secondary visual area.

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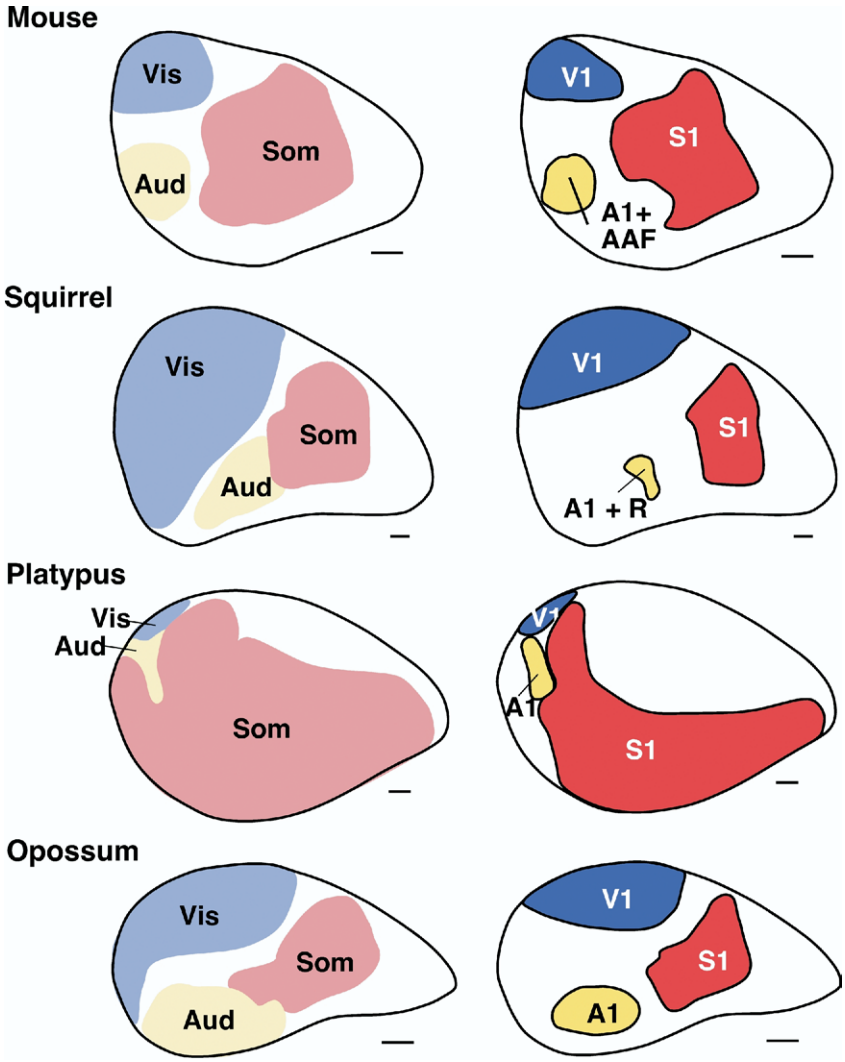


Fig. 1. The organization of sensory domains (left) and primary cortical areas (right) in several species of mammals. Both sensory domains and primary cortical field organization are drawn from electrophysiological studies combined with architectonic analysis, and in most instances, connections. All brains were scaled so that the neocortex is the same size. Note that sensory domain territories (light blue, light yellow and light red), or the amount of cortex devoted to a particular sensory system, vary dramatically in different mammals, even in relatively closely related mammals such as squirrels and mice. These cortical domain territories are directly related to specializations in peripheral morphology and use. For example, squirrels are highly visual arboreal rodents, mice rely on inputs from their vibrissae for most activities, and the platypus is highly reliant on its specialized bill. These behaviorally relevant features of individual species are also reflected in the sizes of cortical fields within a sensory domain (right column). Sensory domains and cortical fields in the mouse are from the present study and Wagor et al. (1980), Woolsey (1967), Woolsey and Van der Loos (1970), and Stiebler et al. (1997); in the squirrel are from Merzenich et al. (1976), Krubitzer et al. (1986), Luethke et al. (1988), Kaas et al. (1989), and Sereno et al. (1991); in the platypus are from Krubitzer et al. (1995); and in the opossum are from Huffman et al. (1999) and Kahn et al. (2000). In this figure and all of the following figures rostral is to the right and medial is to the top. Scale bars=1 mm for all brains. See Table 1 for abbreviations.

in primates (e.g. Krubitzer and Kaas, 1990). Likewise, the relative size of V1 and S1 is different in visual and terrestrial rodents such as squirrels and mice (Fig. 1), and even in different strains of mice (Airey et al., 2005). While it has been proposed that sensory domain allocation, cortical field location, and cortical field size are genetically regulated (e.g. Fukuchi-Shimogori and Grove, 2001; Hamasaki et al., 2004; e.g. Miyashita-Lin et al., 1999), the types of comparative observations described above in mammals suggest that activity from peripheral sensory receptor arrays must also play a role in the actual construction of cortical fields and their connections, since cortical organizational differences so clearly reflect differences in peripheral morphology, receptor type and density, and specialized use.

The idea that sensory driven activity contributes substantially to cortical organization is further substantiated in recent functional imaging studies in blind and deaf humans. In congenitally blind individuals cortex that would normally be activated by visual localization tasks, is activated by auditory localization tasks (Weeks et al., 2000). In congenitally deaf individuals, auditory areas are active during visual and somatosensory tasks (Catalan-Ahumada et al., 1993; Levanen et al., 1998; Finney et al., 2001; see Bavelier and Neville, 2002 for review).

Table 1. Abbreviations

Cortical fields/thalamic nuclei		Body parts	
A1	primary auditory area	Chk	cheek
AAF	anterior auditory field	D2–4	digits 2–4
Aud	auditory cortex	Dig	digits
LGN	lateral geniculate nucleus	FL	forelimb
MGN	medial geniculate nucleus	FP	forepaw
MT	middle temporal visual area	G.HP	glabrous hind paw
PV	parietal ventral area	HL	hindlimb
R	rostral auditory area	HP	hind paw
RS	rhinal sulcus	NAR	nares
S1	primary somatosensory area	Snt	snout
S2	secondary somatosensory area	Trk	trunk
Som	somatosensory cortex	U.Lip	upper lip
V1	primary visual area	Vib	vibrissae
V2	secondary visual area		
Vis	visual cortex		
VP	ventral posterior nucleus		

Because sensory domain assignment and cortical field size vary widely across lineages, and within individuals with different early sensory experience, any theory regarding the development of these features of organization must consider how the mechanisms that gave rise to them could be altered, and thus account for the variability observed across species. The goal of the studies in our laboratory is to determine if the intrinsic and extrinsic developmental mechanisms proposed to generate aspects of cortical field organization are actually operating in naturally evolving systems, and ultimately, if they are mutable by natural selection.

The current investigation is one in a series of studies that we believe will begin to address this issue. Specifically, in this study we used electrophysiological recording techniques combined with myeloarchitectonic analysis (Fig. 2) to examine the organization of the neocortex in two distinct types of congenitally deaf mice, Ca^{2+}-ATPase isoform 2 (PMCA2) and Na^{+}-K^{+}-$2Cl^{-}$ co transporter (NKCC1) knockouts. We chose these two strains of mice for two reasons. First, for the cochlea, both the functional and anatomical consequences of these knockouts have been well documented (Delpire et al., 1999; Dodson and Charalabapoulou, 2001; Flagella et al., 1999; Kozel et al., 1998; Pace et al., 2001; Shull et al., 2003; Street et al., 1998). These previous studies demonstrate that these mice never experience audition because ionic imbalances in the cochlea are incompatible with sensory transduction. Thus, we can determine the contribution of primary afferent input and associated activity during development on aspects of cortical organization in a relatively "normal" genetic cortical environment. Second, mice have a small neocortex. This allows us to explore much of the cortical hemisphere in a single animal and determine the overall effects of peripheral inactivation on sensory domain organization, cortical field size, architecture, and connectivity for most of the cortical fields that occupy the cortical sheet. Most studies of developmental plasticity survey a limited region of the neocortex. The present investigation, on the other hand, is one of the first to explore both the regions of the cortex directly affected by the auditory dysfunction, as well as sensory cortical areas remote from the sensory system directly affected.

EXPERIMENTAL PROCEDURES

NKCC1−/− and PMCA2−/− mice

Both the basolateral NKCC1 and plasma membrane PMCA2 knockout mice were analyzed on a mixed Black Swiss and 129/SvJ background (Flagella et al., 1999; Kozel et al., 1998). NKCC1 knockouts are deaf because the endocochlear potential is abolished and the membranous labyrinth of the inner ear is collapsed; PMCA2 knockouts are deaf because sensory hair cells have abnormal calcium homeostasis. The functional and morphological consequences of these mutations, including auditory brainstem responses (ABRs), have been well documented in both models (Delpire et al., 1999; Dodson and Charalabapoulou, 2001; Flagella et al., 1999; Kozel et al., 1998; Pace et al., 2001; Shull et al., 2003; Street et al., 1998), and it has been conclusively demonstrated that the knockouts are congenitally and profoundly deaf.

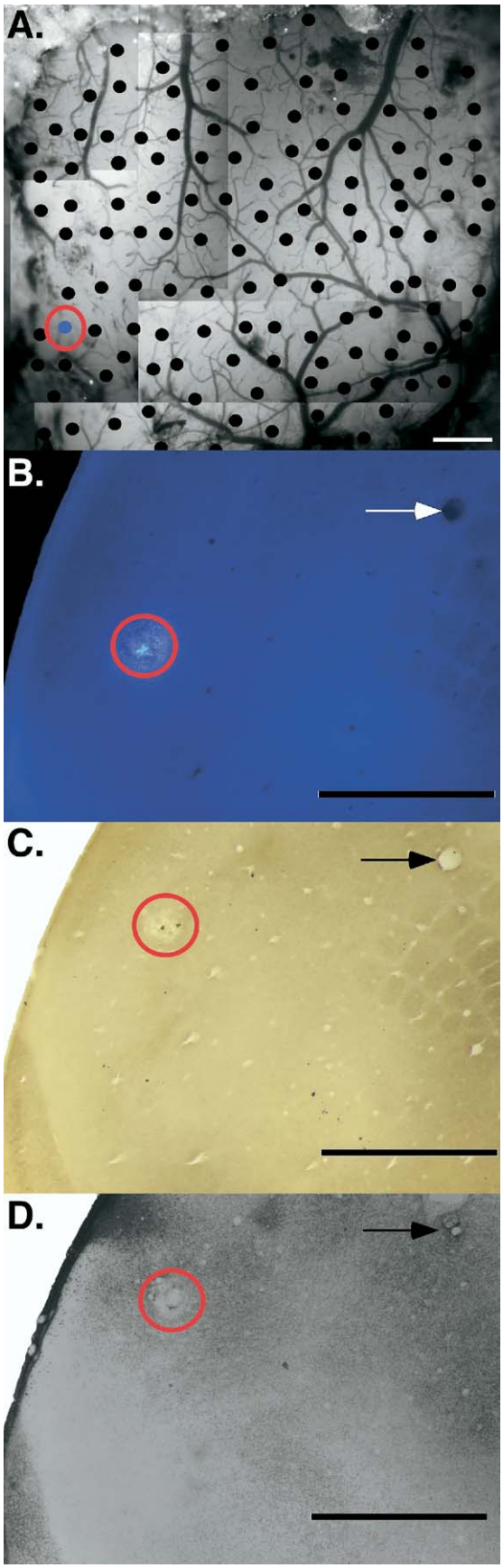
ABR and distortion product otoacoustic emissions (DPOAE)

Mice ranging in age from 4 to 6 months (seven wildtype, four NKCC1−/−, and six PMCA2−/−) were anesthetized with avertin, and ABR measurements were recorded as described by Dou et al. (2000). Briefly, a ground needle electrode and recording needle were placed s.c. in the scalp, and a calibrated electrostatic speaker coupled to a hollow ear bar was placed inside the pinna. Broadband clicks and pure tones (8, 16, and 32 kHz) were presented in the animal's ear in 10 dB increments, from 0 dB SPL to 100 dB SPL. The ABR sweeps were computer-averaged (time-locked with onset of 128–1024 stimuli, at 20/s) from the continuous electroencephalographic activity. The threshold of hearing was defined as the lowest intensity of sound required to elicit a characteristic waveform.

For the DPOAE measurements, the same mice used above were anesthetized with ketamine (95 mg/kg) and xylazine (4 mg/kg). The f_1 and f_2 primary tones were generated by a two-channel frequency synthesizer (Hewlett-Packard 3326A; Hewlett-Packard, Palo Alto, CA, USA), presented through two tweeters (Realistic, Fort Worth, TX, USA), and delivered through a small soft rubber prove tip. Ear-canal sound pressure was measured using a commercial acoustic probe (Etymotic Research $10B^{+}$, Etymotic Research, Elk Grove Village, IL, USA). The ear canal sound pressure was sampled and synchronously averaged ($n=8$) by a digital signal processor for frequencies <20.1 kHz, and by a dynamic signal analyzer (Hewlett-Packard 3561A) for frequencies >20.1 kHz. DP-grams were collected over a range of geometric mean frequencies between 5.6–48.5 kHz ($f_2=6.3$–54.2 kHz), in 0.5-octave intervals at stimulus levels of $L_1=L_2=65$ dB SPL, with $f_2/f_1=1.25$.

Surgical preparation

Electrophysiological recording experiments were performed on 17 young adult mice (nine wildtype, five NKCC1−/−, and three PMCA2−/−). Animals were anesthetized in one of two ways. Some of the animals were anesthetized with isoflurane (2.5%) in an anesthesia induction chamber. Once anesthetized, these animals were tracheotomized and isoflurane was administered through the endotracheal tube. Isoflurane was maintained at 1–2% with 1 L/min oxygen during surgery and throughout the experiment. The other animals were anesthetized with 15–25% urethane administered i.p. at the beginning of each experiment. Throughout all experiments, body temperature, heart rate, and respiration rate were monitored. Hydration was maintained by administering lactated Ringer's solution s.c. every 3–5 h. The only difference observed with the anesthetics used was in the amount



of time the recording session lasted. Recording sessions in which animals were anesthetized with isoflurane generally lasted longer.

After the animals were anesthetized, they were placed in a specially designed holder to immobilize the head. Once secured, the skin was cut, and the skull over one entire hemisphere was removed. A digital image of the entire exposed hemisphere was taken with a Pixera PVC 100c camera (Pixera Corporation; Los Gatos, CA, USA), and the image was printed so that electrode penetrations could be marked relative to blood vessel patterns (Fig. 2A). A small grounding wire was inserted into the opposite hemisphere and held in place with dental acrylic, and the cortex was covered with silicone fluid to prevent desiccation.

Electrophysiological recordings were made with tungsten electrodes (1.0–5.0 M Ω at 1 kHz) designed to record extracellularly from single neurons and neuron clusters. Neural activity was amplified, filtered, viewed on an oscilloscope, and heard through a loudspeaker. Electrode penetrations were made at multiple sites to survey a large portion of the neocortex, and each penetration was considered a single recording site. Stimulus preferences were recorded at all sites, and receptive fields were identified for all sites at which neurons responded to somatosensory stimulation. Because we utilized multiunit recording techniques, for bimodal responses, it could not be determined whether individual neurons were bimodal or unimodal. The goal of these experiments was not to determine the detailed topographic organization of any one field; this has already been done for the V1 (Dräger, 1975; Wagor et al., 1980), the primary and secondary somatosensory areas (S1 and S2; Carvell and Simons, 1986; Woolsey, 1967; Woolsey and Van der Loos, 1970), and for the primary auditory area and surrounding auditory fields in mice (A1 and AAF; Stiebler et al., 1997). Thus, the topographic organization, location, and architectonic/histochemical appearance of V1, S1, S2, A1, and AAF have been well established for the mouse. Our objective was to relate the primary cortical fields to architectonic boundaries in tangentially sectioned cortex, and to determine the total extent of particular sensory domains. Thus, the stimuli used were relatively simple and could be applied rapidly. These procedures allowed us to survey the entire extent of the neocortex, often in a single animal. Visual stimuli included full-field flashes, bars, and circles of light either moving through the receptive field or turned on and off within the receptive field. Auditory stimuli consisted of high and low frequency noise. Somatic stimuli were applied to the skin with

Fig. 2. Methods of reconstructing electrophysiological recordings results and relating these to architectonic boundaries. A digital image of the exposed neocortex is made prior to the commencement of electrophysiological recordings (A). An electrode is placed at a number of sites in the cortex and these sites are marked on the digital image of the brain (black dots in A). Neural activity is recorded at each site, and the modality of the stimulus that activated these neurons is noted, as is the receptive field location for neurons that respond to somatosensory stimuli. Some recording sites are marked by inserting a blue fluorescent dye (Fast Blue; red circles in A–D) at a particular site for later identification in histologically processed tissue (B–D). Because the electrode is dipped in the fluorescent dye, the sites marked appear as large fluorescent blue deposits (B). Histological sections are drawn using a camera lucida, and the outline of sections, blood vessels (arrows in B–D), and blue dye deposits (identified with red circles in B–D) are all marked. By matching probe locations, blood vessels, and outlines of the tissue, CO (C) and myelin (D), boundaries can be directly related to electrophysiological recording results (see Experimental Procedures). In this way, a comprehensive reconstruction of the neocortex is generated. Images B, C and D are of cortex that has been flattened and cut tangential to the cortical surface and are shown at the same magnification. The digital image of the exposed neocortex is taken at a lower magnification so that all of the recording sites can be viewed and identified with respect to the vascular pattern of the neocortex during electrophysiological recordings. Scale bars=1 mm.

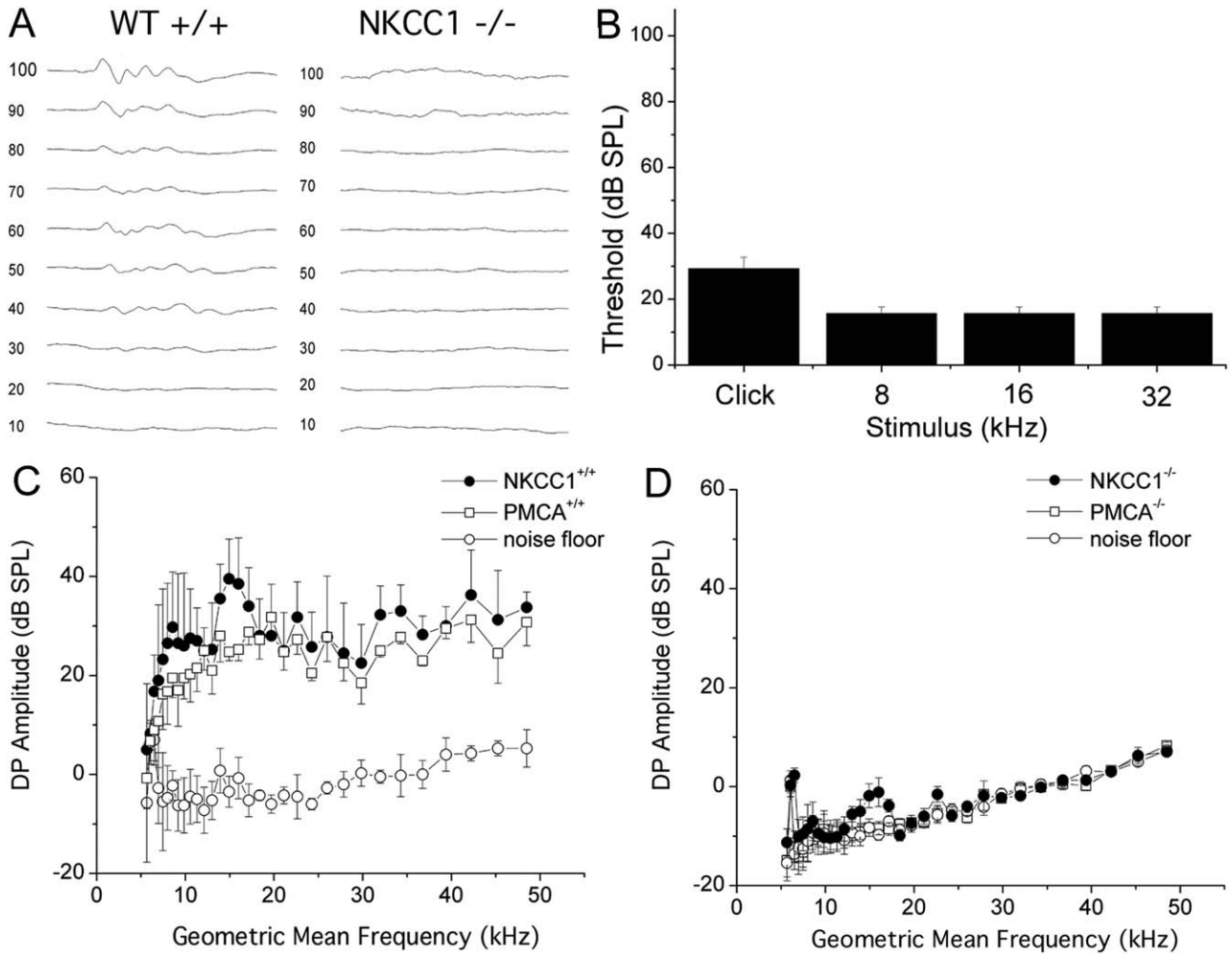


Fig. 3. (A) ABR thresholds from the right ears of wildtype (left panel in A), and homozygous NKCC1−/− mice (right panel in A) that are 4–6 months old. Similar null traces were obtained for homozygous PMCA2−/− mice. The sound pressure levels in dB of broadband clicks (0.1 ms) delivered to the ear are indicated on the left side of the traces. Broadband click responses from wildtype mice are normal while no response was observed from any of the null mutants mice examined. (B) ABR thresholds for wildtype mice (solid black) in response to broadband clicks and 3-ms pure tones of 8, 16, and 32 kHz. Threshold in dB SPL is marked on the *y* axis. NKCC1−/− and PMCA2−/− mice did not respond to the same stimuli and, hence, are not depicted in panel B. (C) Mean DP-grams from 4–6 month old wildtype mice obtained by measuring the levels of the $2f_1$-f_2 DPOAE over a geometric-mean frequency range from 5.6–48.5 kHz, using an f_2/f_1 of 1.25, and primary tone stimuli at $L_1=L_2=75$ SPL. (D) Shows the corresponding data obtained from age-matched littermates of the null mutant mice. Clearly, $PMCA^{-/-}$ and $NKCC1^{-/-}$ mice yielded no significant DPOAEs at low and high frequencies as compared with the wildtype mice. These data indicate that both PMCA2−/− and NKCC1−/− mice are profoundly deaf due to cochlear dysfunction.

a fine probe or brush for cutaneous receptors. To stimulate deep receptors, taps to the body, muscle manipulation, and displacements of limbs and joints proved effective. In both the wildtype and mutant mice, all stimuli were tested at every recording site. Throughout the experiments, slight changes in body temperature and anesthetic levels resulted in a loss of responsiveness of cortical neurons; these sites are marked with X's in the figures. During the electrophysiological recording session, selected sites were marked using a fluorescent dye (7% solution of Fast Blue; Sigma Aldrich) for later identification in histologically processed tissue (Fig. 2A and B). All procedures were approved by the Animal Use and Care Administrative Committee of the University of California, Davis, and conformed to NIH guidelines.

Histological tissue preparation

After electrophysiological recordings were complete, the animal was killed, and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in phosphate buffer (pH 7.2) and then 4% paraformaldehyde in 10% sucrose phosphate buffer (SPB). The cortex was removed from the brainstem and thalamus, flattened between glass slides, and left to soak overnight in 30% SPB. Cortex was cut tangentially on a freezing microtome into 25–30 μm thick sections, and was processed for cytochrome oxidase (CO) (Wong-Riley, 1979) and myelin (Gallyas, 1979). In addition to the animals used in electrophysiological recording experiments, seven additional normal mice, five additional NKCC1−/− mice, and two additional PMCA2−/− mice were used for histological processing. We chose these cases based on the clarity of the myelin stains and the confidence with which we could assign primary cortical field boundaries. In these animals, brains were cut as described above and stained for myelin so that measurements of primary sensory areas could be made, and differences in cortical field size could be statistically analyzed (see below).

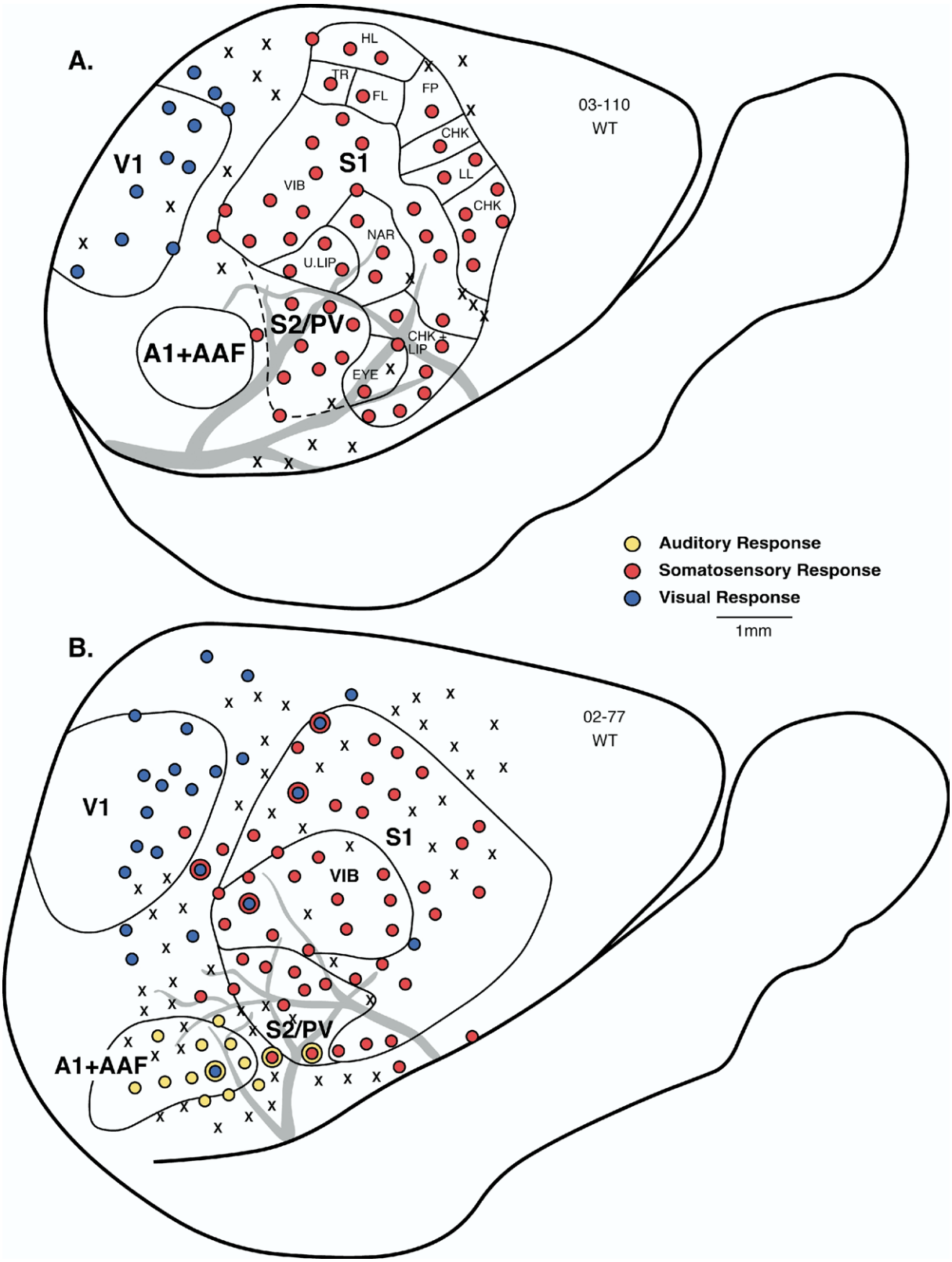


Fig. 4. Reconstructions of maps of the neocortex in two wildtype mice (A and B) in which a large expanse of cortex was explored using electrophysiological recording techniques. Neurons in S1 responded to cutaneous stimulation of the contralateral body (red dots). Within S1, a topographic representation of the contralateral skin surfaced was organized from tail and hindlimb representations medially to forelimb and face laterally. Neurons in V1 responded almost exclusively to visual stimulation of the contralateral hemifield (blue dots), and neurons in area A1+AAF

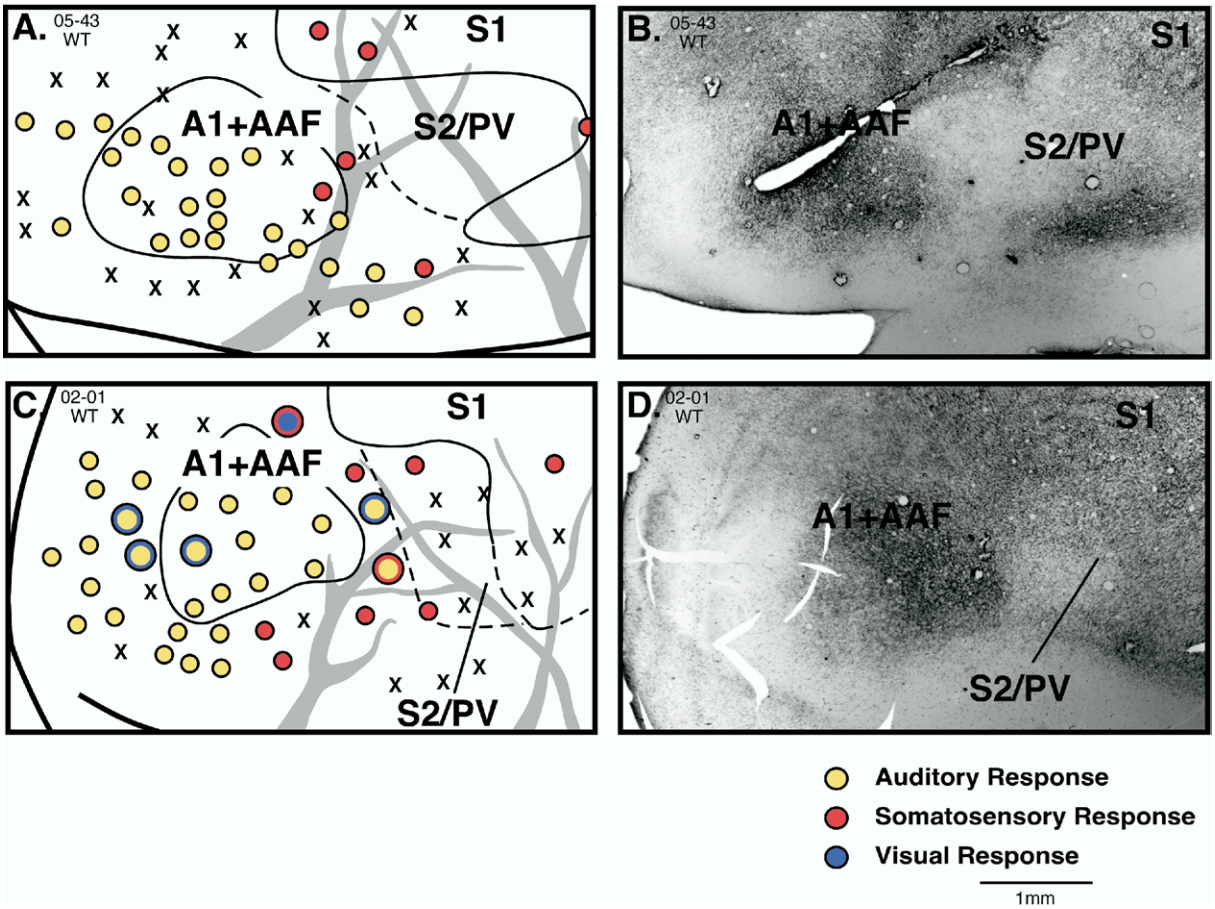


Fig. 5. Reconstructions of maps of auditory cortex (A and C) and corresponding myelin-stained sections (B and D) in two wildtype mice. Cortical area A1+AAF stains darkly for myelin and is located just caudal to one of the two large blood vessels (gray) originating from the location of the rhinal sulcus. As in the cases illustrated in the previous figures, neurons in A1+AAF were responsive to pure auditory stimulation, except at one site in each case. Neurons caudal (A and B) and lateral (A) to A1+AAF were also responsive to auditory stimulation. Several sites in cortex surrounding A1+AAF contained neurons that were responsive to auditory+visual stimulation, auditory+somatosensory stimulation, and visual+somatosensory stimulation. A few sites lateral to A1+AAF in case 02–01 (C) contained neurons responsive to pure somatic stimulation. Architectonic boundaries drawn in the cortical reconstructions in A and C are from the entire series of myelin-stained sections, and are not drawn only from the single sections (B and D) shown in this figure. Thus, the correspondence between the reconstructions in figures A and C and myelin stains in B and D is very good, but these figures do not match exactly. Conventions as in previous figures.

Data analysis

Data analysis consisted of two stages. In the first stage, all electrode penetrations, including those marked with fluorescent tracers (Fig. 2B), and cortical vasculature were traced directly from the digital image of the neocortex made during the experiment. Stimulus preferences for each site were marked. Neurons with similar modality preferences were grouped together and lines were drawn around these sites. The lines interpolated between these sites and adjacent sites in which neurons had a different modality preference. At some locations, neurons responded to more than one modality of stimulation. If this was the case, multimodal sites were grouped together. For the somatosensory system, neurons with receptive fields on the same body part were grouped together, and lines were drawn around these sites midway between them and adjacent sites representing a different body part. In the second stage of analysis, architectonically defined borders were related to electrophysiological results by overlaying the tracing containing the electrode penetrations, probes marked with fluorescent tracer, blood vessels, and sensory maps

responded to auditory stimulation (yellow dots). Although there is variation in the vascular pattern of the neocortex, A1+AAF are consistently located just caudal to one of two large blood vessels (light gray) originating from the location of the rhinal sulcus. These regions were directly related to architectonically defined cortical boundaries (solid black lines) determined using the entire series of sections stained for myelin. The method of matching data sets is depicted in Fig. 2 and is explained in the Experimental Procedures. Neurons outside of the primary areas were sometimes responsive to sensory stimulation in our preparation, but the responsiveness was less robust than in primary fields. In case 02–77 (B), at several sites neurons were responsive to bimodal stimulation (blue and red circles and blue and yellow circles), but these sites were limited and occurred either in non primary fields, or within 500 μm of the boundary of a primary field. Finally, sites marked with X's contained neurons that were unresponsive. This was likely the result of fluctuations in anesthetic level and/or body temperature. The light gray indicates blood vessels taken directly from the digital image of the brain upon which electrode penetrations were marked. See Table 1 for abbreviations. Rostral is to the right and medial is to the top. Conventions as in previous figures.

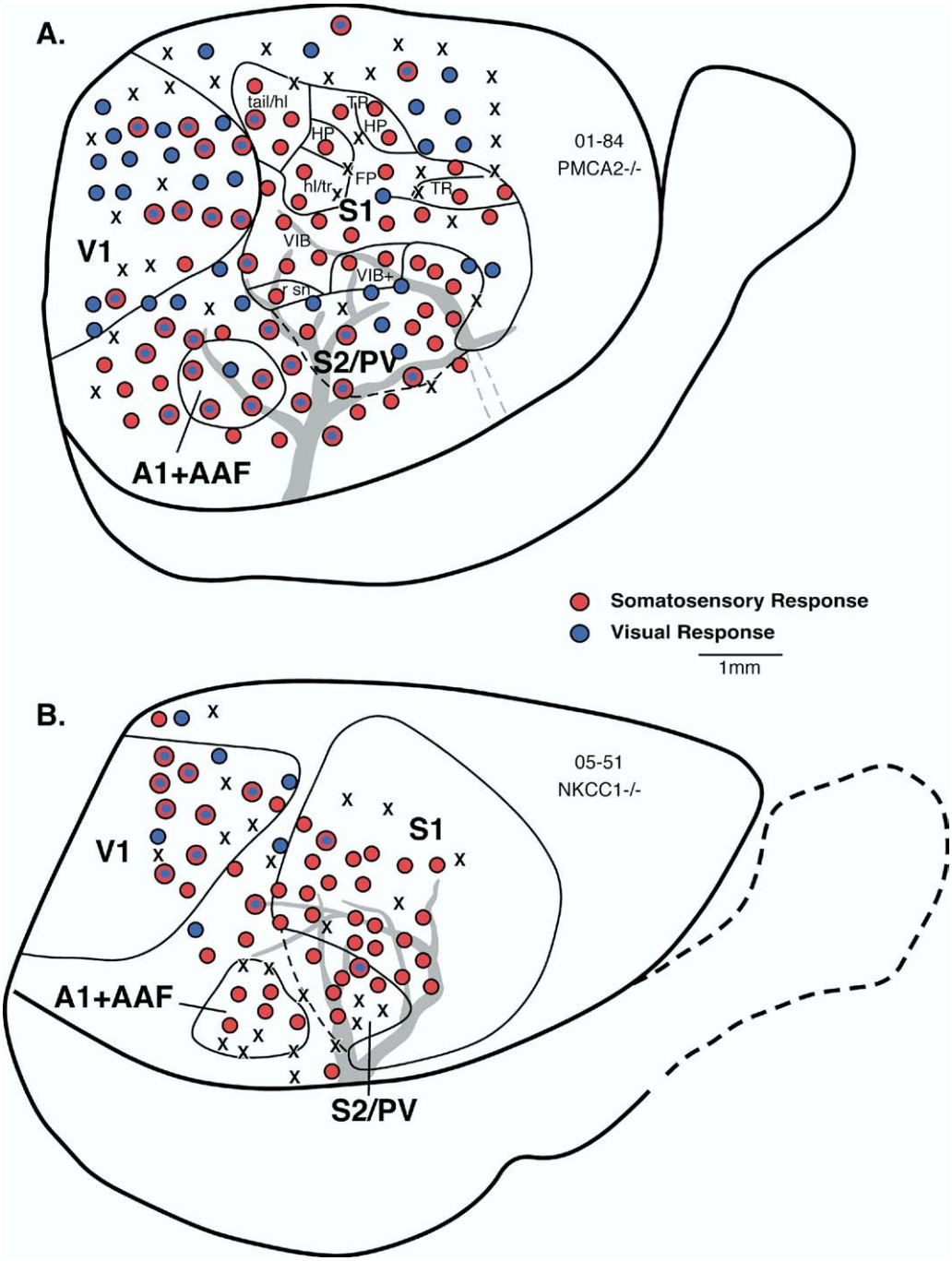


Fig. 6. Reconstructions of maps of the neocortex in the congenitally deaf PMCA2−/− (A) and the NKCC1−/− mouse (B). As in wildtype mice, neurons in S1 responded to cutaneous stimulation of the contralateral body surface, and a topographically organized representation of the body was observed in S1 (A). While neurons in V1 responded to visual stimulation of the contralateral hemifield, a larger proportion of neurons responded to visual+somatosensory stimulation compared with normal animals. Neurons in area A1+AAF responded to somatosensory (B), visual, or both visual and somatosensory stimulation (A). As in wildtype animals, electrophysiological recordings were related to architectonically defined fields (solid lines), and are located just caudal to one of two large blood vessels. Conventions as in previous figures and abbreviations in Table 1.

onto histologically processed sections drawn to exactly the same scale using a camera lucida (Fig. 2). For the determination of boundaries using myelin-stained and CO-stained sections, the entire series of sections was drawn and included the fluorescent probes (for electrophysiological mapping cases), the outline of the sections, blood vessels, and tissue artifacts. It should be noted that the boundaries of all cortical fields were not always visible in a single section (e.g. Figs. 2, 5 and 7). However, by analyzing the entire series of sections, cortical field boundaries, particularly for primary fields, could be accurately and reliably drawn.

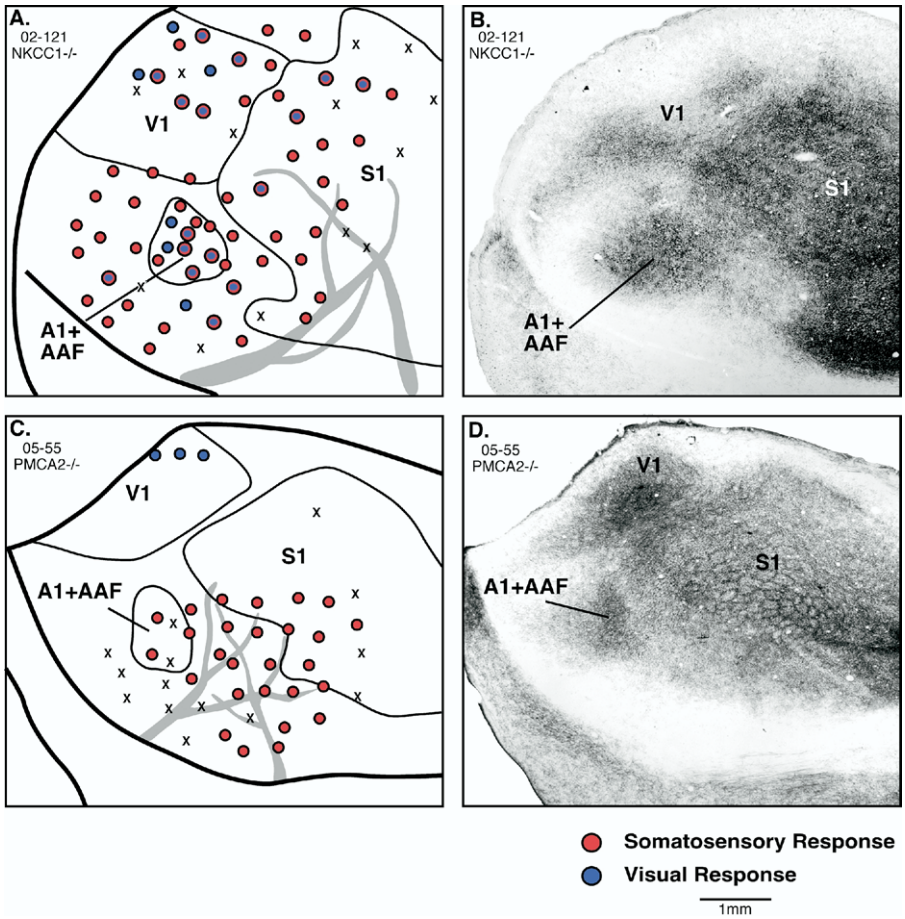


Fig. 7. Reconstructions (A and C) and myelin stains (B and D) of sensory cortex in congenitally deaf mice. As in the previous cases, V1 (A) contains neurons responsive to visual, somatosensory, or both visual and somatosensory stimulation. In case 05–55, only three recording sites were obtained in V1 and all of these responded to pure visual stimulation. In both cases, neurons in A1+AAF responded to visual, somatosensory, or both visual and somatosensory stimulation (A), or to somatosensory stimulation (C). Neurons in cortex caudal to A1, responded to somatosensory stimulation (except for one site). In wildtype mice, this cortex contained neurons responsive to auditory stimulation (see Fig. 5). As in wildtype mice, architectonically defined A1+AAF stains densely for myelin and is located caudal to one of two large blood vessels. Architectonic boundaries drawn in the cortical reconstructions in A and C are from the entire series of myelin-stained sections, and are not drawn only from the single sections (B and D) shown in this figure. Thus, the correspondence between the reconstructions in figures A and C and myelin stains in B and D is very good, but these figures do not match exactly. Conventions as in previous figures.

For analysis of cortical field size in both normal and deaf mice the following procedures were used. First, the section where the area of the neocortical sheet was the greatest in size was determined. In all cases, this section was from middle cortical layers. After this section was identified, the outline of the neocortex, blood vessels, pyriform cortex, olfactory bulb, and cortical field boundaries was drawn with a camera lucida. By matching blood vessels and other landmarks (see above), cortical field boundaries were identified using the entire series of sections and collapsed onto this baseline section. For this analysis, we drew only the S1, V1, and primary auditory area+anterior auditory field (A1+AAF). These cortical fields were readily identified in both normal and deaf mice. Reconstructions of cortical field boundaries were scanned into a computer. The area of the entire neocortex and the area of each field (S1, V1, and A1+AAF) were calculated in mm^2 on a PC computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/ij). All reconstructions and measurements were done under blind conditions.

A one-way analysis of variance was used to examine the differences in the percentage of cortex occupied by each area (S1, V1, and A1+AAF) between genotypes (wt, NKCC1−/−, and PMCA2−/−. Post hoc analyses were used when applicable. Analyses were conducted using Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA). For analysis of the modality preferences for responsive neurons, proportions were calculated by dividing the number of neurons responding to pure or bimodal stimulation by the total number of responsive neurons within the architectonically defined primary area.

RESULTS

ABR and DPOAE

The anatomical, functional, and behavioral consequences of both the PMCA2−/− and NKCC1−/− knockouts have been well documented for five to eight week old mice (Delpire et al., 1999; Dodson and Charalabapoulou, 2001; Flagella et al., 1999; Kozel et al., 1998; Pace et al., 2001; Shull et al., 2003; Street et al., 1998). We performed ABR's and DPOAE's on our adult animals, and in two of the deaf mice (cases 05–51 and 05–55) electrophysiological recording data were gathered as well. ABR's reflect the

integrity of inner hair cell and/or auditory brain stem function, while DPOAE's reflect outer hair cell function. Together, these tests demonstrate the auditory sensitivity of the animal for different frequencies at various sound pressure levels (measured in decibels, dB), and help determine the locus of the loss (i.e. the cochlea, rather than the CNS). The results of our investigation were similar to those described previously by Kozel et al. (1998) and Flagella et al. (1999). Briefly, wildtype mice exhibit a characteristic ABR waveform at sound pressure levels ranging from 10–100 db for frequencies of 8, 16, and 32 kHz, and for broadband clicks (Fig. 3A and 3B). Neither the NKCC1−/− nor the PMCA2−/− mice exhibited a characteristic ABR. Rather, for all frequencies and intensities tested, these mice were demonstrated to be deaf (Fig. 3A). Likewise, while the DPOAE's for wildtype mice were normal (Fig. 3C), the distortion product amplitude for the deaf mice was at the level of the noise floor (Fig. 3D). This result indicates that the etiology of the hearing deficit is the result of inner ear malfunction.

The organization of sensory cortex in normal mice

For these experiments, we noted several characteristics for neurons at all recording sites. These include the level of responsiveness to sensory stimulation (e.g. unresponsive vs. highly responsive), responsiveness to pure sensory versus mixed sensory stimulation, and for somatosensory cortex, the receptive field size and location. Using this information, combined with architectonic analysis, we found that a number of aspects of cortical organization were similar to those described previously for visual, somatosensory, and auditory cortex of the mouse. For somatosensory cortex, 84.47% of neurons in S1 (Table 2) were responsive exclusively to cutaneous stimulation of the contralateral body, and a complete, or nearly complete, representation of the body surface was identified (Fig. 4A). S1 occupied 32.71% of the cortical sheet (Table 2, myeloarchitectonic measurements). The mediolateral organization of this field was like that described previously for the mouse (Woolsey, 1967; Woolsey and Van der Loos, 1970), as well as other mammals (Johnson, 1990 for review), in that the tail and foot were represented most medially in the field, followed by representations of the forepaw, face, and vibrissae laterally (Fig. 4A). As in a number of other rodents, and as previously described for the mouse, the representation of the whiskers, termed the barrel field, occupied a large portion of the S1 cortex. Receptive fields for neurons in S1 were relatively small and were limited to small portions of the body such as the nares, or a small portion of the face. We did not attempt to delineate individual vibrissae representations in these experiments. Finally, this entire representation was coextensive with a unique architectonic field as described below.

A small field was identified just caudolateral to S1 and contained an additional representation of the body surface (Fig. 4). The functional organization of this region of cortex which contains both S2 and PV, has been described in mice (Carvell and Simons, 1986), and in other rodents such as rats (Remple et al., 2003) and squirrels (Krubitzer et al., 1986) (Table 1 for abbreviations). In the present investigation, we did not attempt to distinguish between S2 and PV and thus term this region S2/PV. Neurons in S2/PV responded well to unimodal somatosensory stimulation of the contralateral body surface, and receptive fields for neurons at these sites were large relative to receptive fields for neurons in S1, and encompassed large portions of the body.

We did not attempt to map receptive fields or delineate the topography of V1 since it has been well characterized in mice (Dräger, 1975; Wagor et al., 1980), other rodents (Kaas et al., 1989; Paolini and Sereno, 1998; Sereno et al., 1991), and other mammals (Kaas, 1980; Rosa and Krubitzer, 1999 for review). However, we could readily distinguish V1 from surrounding cortex in two ways. First, 97.92% of neurons in V1 were highly responsive to unimodal visual stimulation of the contralateral hemifield (Table 2). Second, this region of cortex was co-extensive with a unique architectonic appearance that could be readily and consistently identified (see below). V1 occupied 8.96% of the cortical sheet (Table 2).

Table 2. Mean percentages of modality of recording sites within primary fields and of cortical field size

Genotype	Cortical area	Percentage of cortical sheet			Response modality					
		n	M	SD	Aud	Som	Vis	Aud+Som	Aud+Vis	Som+Vis
WT	A1+AAF	15	4.35	0.90	90.65	1.85	0.00	0.00	7.50	0.00
	S1	14	32.71	2.32	0.00	84.47	9.09	4.17	0.00	2.27
	V1	14	8.96	2.31	0.00	2.08	97.92	0.00	0.00	0.00
	Non-primary	N/A	N/A	N/A	32.53	42.10	18.29	1.62	3.21	2.25
NKCC1$^{-/-}$	A1+AAF	8	2.80	0.67	0.00	68.17	13.65	0.00	0.00	18.18
	S1	8	34.78	2.70	0.00	85.96	0.00	0.00	0.00	14.04
	V1	8	13.06	1.93	0.00	31.02	21.54	0.00	0.00	47.44
	Non-primary	N/A	N/A	N/A	0.00	84.89	7.69	0.00	0.00	7.42
PMCA2$^{-/-}$	A1+AAF	6	2.70	0.69	0.00	50.00	10.00	0.00	0.00	40.00
	S1	6	33.09	4.04	0.00	70.52	12.05	0.00	0.00	17.43
	V1	6	12.28	2.61	0.00	1.07	82.58	0.00	0.00	16.35
	Non-primary	N/A	N/A	N/A	0.00	68.34	17.58	0.0	0.00	14.08

Aud, auditory; M, mean; n, number of hemispheres; N/A, not applicable; SD, standard deviation; Som, somatosensory; Vis, visual.

A small region of cortex in the caudotemporal pole, immediately superior to the rhinal sulcus (Figs. 4 and 5) contained neurons that responded almost exclusively to pure auditory stimulation (90.65%; Table 2). Previous studies in the house mouse (Stiebler et al., 1997) (*Mus musculus*) and other rodents (Luethke et al., 1988; Merzenich et al., 1976; Ohl et al., 2000a,b; Rutkowski et al., 2000; Zhang et al., 2002, 2003) demonstrate that this region of cortex contains the A1, as well as the anterior AAF, also termed R (termed A1+AAF in the present study), and that neurons in A1+AAF respond exclusively to auditory stimulation. As with V1 and S1, area A1+AAF is coextensive with a unique architectonic appearance (Fig. 5). This field occupied 4.35% of the cortical sheet (Table 2; see below). Since we were primarily interested in delineating the sensory domains and primary cortical fields, we did not attempt to produce tonotopic maps of auditory cortical areas.

The organization of sensory cortex in congenitally deaf mice

Using similar electrophysiological methods to those used for mapping normal mice, the organization of sensory cortex was explored and related to architectonic boundaries in deaf mice (Figs. 6 and 7). There were no differences in neural responsiveness in that the majority of neurons recorded in A1+AAF, V1 and surrounding cortex had moderate (25%) to very good (43%) responsiveness, while about 32% responded weakly to sensory stimulation in deaf mice. The receptive field size for neurons responsive to somatosensory stimulation in both groups of deaf mice was generally similar to that in normal mice, but for some recording sites, neurons had split receptive fields on portions of the face and portions of the body. There were several important differences observed between deaf and normal mice. The first is that cortex that would normally contain neurons that respond to auditory stimulation (Figs. 6 and 7), contained neurons that responded to somatosensory (68.17% and 50.00%), visual (13.65% and 10.00%), or somatosensory+visual stimulation (18.18% and 40.00%; Table 2). Receptive field locations for neurons in reorganized auditory cortex are given in Fig. 8. The majority (69%) of receptive fields in re-organized A1 were on the face, with fewer receptive fields on other body parts such as the hindlimb, trunk and tail (27%). At a few sites (4%) receptive fields were split and were on the face and some other body part (Fig. 8). The second observation was that cortex that would normally contain neurons that respond to unimodal visual stimulation (V1), contained neurons that responded to somatosensory (31.02% and 1.07%), visual (21.54% and 82.58%) or visual+somatosensory stimulation (47.44% and 16.35%; Table 2). Receptive field locations in re-organized visual cortex are given in Fig. 8. As with auditory cortex, the majority of somatic receptive fields in V1 in deaf mice were on the face (58%), while fewer receptive fields (24%) were on other body parts. About 18% of receptive fields for neurons in V1, which responded to somatic stimulation, had split receptive fields on both the face and some other body part. Thus, not only was the deprived auditory cortex re-organized, but visual cortex

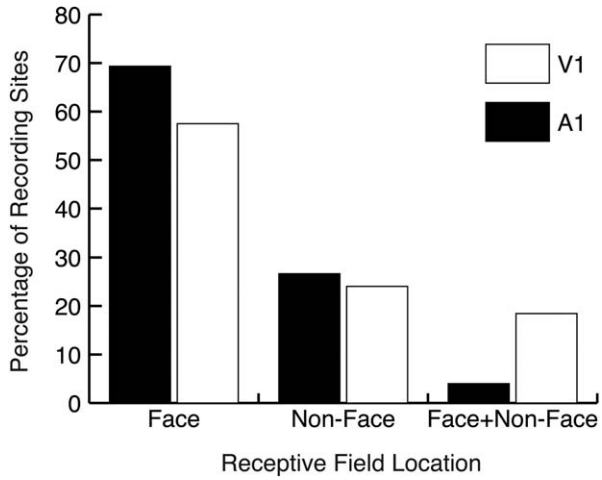


Fig. 8. A histogram showing the percentage of recording sites A1 and V1 in deaf mice that had receptive fields on the face, non-face and face+non-face locations of the body. These percentages were not derived from neurons at all recording site, only those that contained neurons that were responsive to somatic stimulation. Note that for both re-organized V1 and A1, the majority of recording sites (58% and 69% respectively) contained neurons with receptive fields on the face. A lower percentage of neurons had receptive fields on non-face regions of the body or on both face and non-face regions of the body. For simplicity we grouped together receptive fields on the vibrissae, snout, lips or other parts of the face as "face" receptive fields. Likewise receptive fields on the tail, hindpaw and trunk were termed "non-face" receptive fields.

appeared to undergo re-organization as well. This effect appeared greater for the NKCC1−/− mouse than for the PMCA2−/− mouse, and this may be due to the different behavioral deficits induced by each mutation. For example, NKCC1−/− mice have no behavioral deficits or mild ones such as head bobbing and running in tight circles. PMCA2−/− mice can vary in their behavioral deficits from mild deficits (like NKCC1−/−) to profound deficits such as lack of control over their head movements, lack of hindlimb function, and overall lack of movement. The third observation was that the size of V1 and area A1+AAF was different in the congenitally deaf mice than the wildtype mice (Table 2, Fig. 9). Finally, the organization of S1 was similar to that described for wildtype mice in that neurons in S1 were predominantly unimodal in both NKCC1−/− and PMCA2−/− mice (85.96% and 70.52%, respectively, and the relative size of S1 (NKCC1−/−=34.78% and PMCA2−/−=33.09%) was similar to that of the wildtype mice (WT=32.71%). The body map in S1 was much like that described for normal animals (compare Fig. 4A with Fig. 6A). As noted below, the architectonic appearance of primary cortical areas in these animals was similar to that described for wildtype animals.

Architecture of cortical fields and their relation to electrophysiological recording results in wildtype and congenitally deaf mice

In both normal and congenitally deaf mice, electrophysiological recording sites were directly related to cortical myeloarchitectonic borders (see methods; Figs. 2, 5 and 7). In

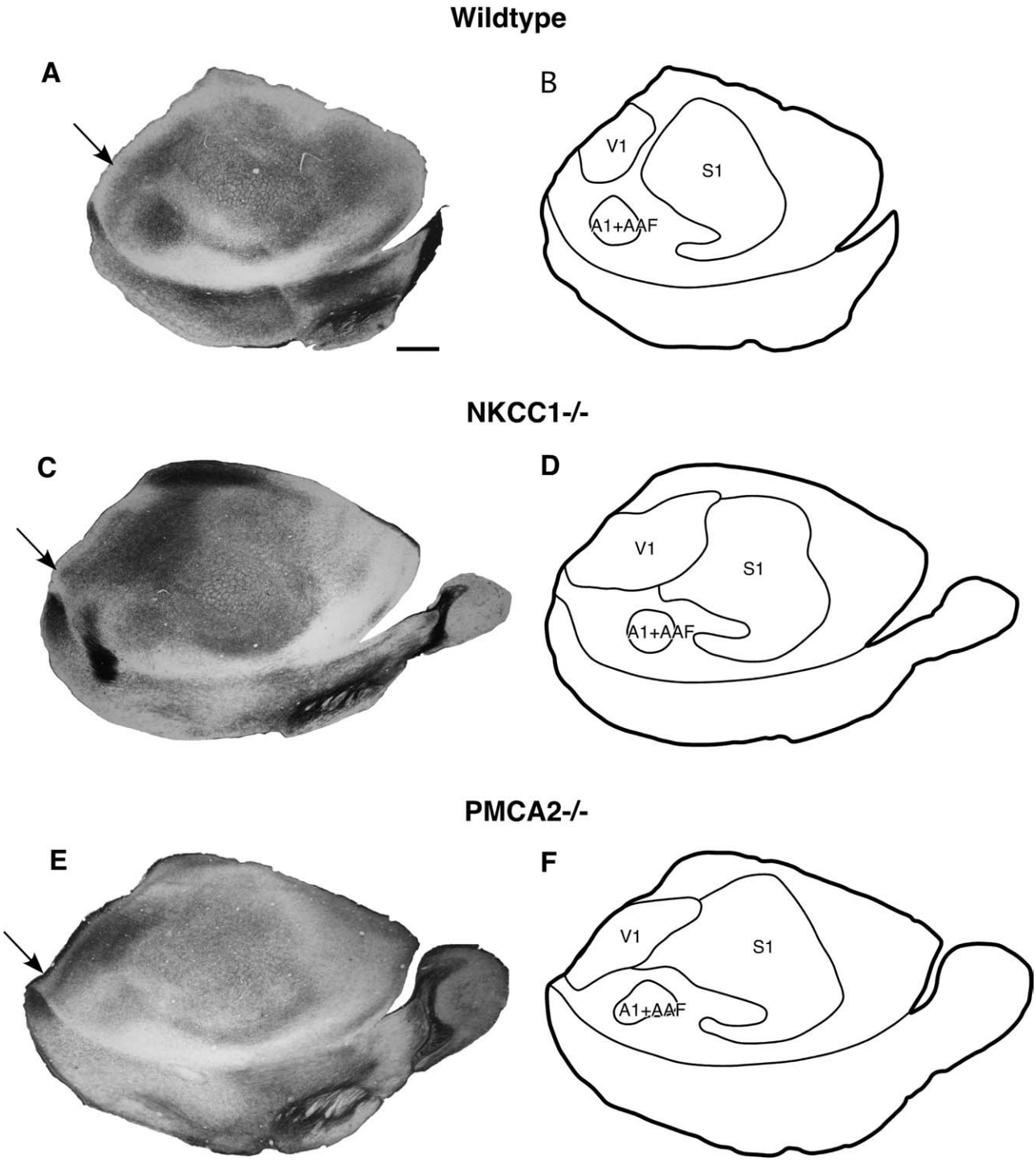


Fig. 9. Myeloarchitecture of cortex that has been flattened and cut parallel to the cortical surface in wildtype (A), NKCC1−/− (C), and PMCA2−/− (E) mice. The boundaries drawn for these cases in B, D and F are done by examining the entire series of sections because all of the cortical field boundaries are often not visible in a single section. In these particular sections, several cortical field boundaries are readily distinguished. Area A1+AAF is clearly visible as a moderate to darkly myelinated circle in the temporal pole of cortex. S1 is a large moderately myelinated field, and V1 is a darkly myelinated field located in the occipital pole of the neocortex. In C and E the lateral edge of striate cortex (V1) is clearly visible (arrow), and is in a location that is only a few hundred microns from the rhinal sulcus. This is remarkably different than the lateral boundary of V1 in normal animals (see arrow in A). Conventions as in previous figures.

normal mice, cortex that contained neurons that responded well to auditory stimulation was coextensive with a darkly myelinated circle of cortex in the temporal lobe termed A1+AAF (Figs. 4, 5, 9A and 9B). V1 was identified as a darkly myelinated wedge of cortex at the caudal occipital pole of the cortex in which neurons responded to

visual stimulation. S1 was coextensive with a large darkly myelinated area in the parietal region of cortex in which neurons responded to tactile stimulation, and contained a complete representation of the contralateral body surface. In wildtype and congenitally deaf mice, the barrel fields could be readily identified in S1 in cortex reacted for CO and myelin, (Fig. 2C, 7 and 9), as described in previous studies. In deaf mice, all of the primary areas were readily visible (Figs. 7 and 9), but there was a de-correlation between function and architecture for both A1+AAF and V1 (Figs. 6 and 7).

In this investigation, we were also interested in whether the size of cortical fields was different in wildtype and congenitally deaf mice (NKCC1−/− and PMCA2−/−). To determine this, cortical hemispheres in wildtype mice (n=10) and congenitally deaf mice (n=6 NKCC1−/− and four PMCA2−/−) were stained for myelin and/or CO and the boundaries of primary areas (V1, S1, and A1+AAF) were determined in a blind analysis (Fig. 9, see Experimental Procedures). We did not separate NKCC1+/+ from PMCA2+/+ mice since these mice were the same strain and were housed under identical conditions. The size of each primary field was calculated as a percentage of the entire cortical sheet (Table 2). This eliminated the confound of differences in body weight/brain weight across animals. A one-way ANOVA indicated two significant differences in the relative size of the primary cortical fields in wildtype and deaf animals. First, area A1+AAF was significantly different in size in wildtype versus congenitally deaf mice, $F(2, 26)=14.21$, $P=0.001$ (Fig. 4). A post hoc analysis (Scheffé test) indicated that area A1+AAF was significantly larger in the wildtype mice (M=4.35%, S.D.=0.90) than in the NKCC1−/− (M=2.8%, S.D.=0.67) and PMCA2−/− (M=2.70%, S.D.=0.69) mice, $P=0.001$. No significant difference in the percentage of the cortical sheet occupied by A1+AAF was indicated between the NKCC1−/− and PMCA2−/− mice. Second, there was a significant difference in the size of V1 in wildtype and congenitally deaf mice, $F(2, 25)=9.8$, $P=0.001$ (Fig. 4). A post hoc analysis (Scheffé test) indicated that V1 was significantly smaller in the wildtype mice (M=8.96%, S.D.=2.31) than in the NKCC1−/− (M=13.06%, S.D.=1.93) and PMCA2−/− (M=12.28%, S.D.=2.61) mice, $P=0.020$. No significant difference in the percentage of the cortical sheet occupied by V1 was indicated between the NKCC1−/− and PMCA2−/− mice. Finally, no significant differences in the percentage of the cortical sheet occupied by S1 area were observed between genotypes (wildtype, NKCC−/−, and PMCA−/−), $F(2, 25)=1.40$, $P=0.266$.

The combined electrophysiological recording results and architectonic analysis led to four main observations. First, in wildtype mice, there was a strong correlation between myeloarchitectonically and electrophysiologically defined primary sensory areas. Second, electrophysiologically identified cortical domain territories shift dramatically in congenitally deaf mice in that auditory cortex is taken over by the visual and somatosensory system. Third, V1 was functionally reorganized. Fourth, although the architectonic appearance of the primary cortical areas S1, V1, and area A1+AAF was the same in wildtype and congenitally deaf mice, area A1+AAF was significantly smaller and V1 was significantly larger in congenitally deaf mice than in wildtype mice.

DISCUSSION

Our results are the first demonstration that congenital loss of sensory driven activity results in extensive multisensory plasticity and a re-specification of cortical fields. All of what would normally be auditory cortex is taken over by the visual and somatosensory system. Our study also demonstrates that dysfunction or loss of one sensory system not only has a large effect on the system in question, but on other sensory systems as well. For example, V1 has increased in size and the modality preference of neurons in V1 has been dramatically altered. We also demonstrate that it is possible to de-correlate functional and anatomical maps of the neocortex, as has been observed in naturally evolving systems. Finally, when data sets are compared from studies that used a variety of techniques to examine aspects of cortical field development, it becomes clear that alterations in fundamental features of cortical organization, such as the size of a cortical field, can be achieved not only by altering genes intrinsic to the neocortex, but by altering sensory driven activity in developing animals. Below we first describe previous studies in which similar techniques were used to explore and subdivide sensory neocortex in mice. We then discuss results of different studies that indicate that genes or activity can modify the same aspect of cortical organization such as sensory domain allocation, cortical field size, and aspects of thalamocortical connectivity. While the developmental mechanisms that give rise to this phenomenon are not reported in this study, in the final portion of this discussion we explore potential mechanisms that may contribute to the changes in organization that we observe, and that might operate naturally in evolution.

Organization of sensory neocortex in normal mice

There are only a few studies in mice which used techniques similar to those used in the present study to examine the detailed organization of sensory cortical fields including S1 (Woolsey, 1967), V1 (Dräger, 1975; Wagor et al., 1980), A1, AAF, UF (Stiebler et al., 1997), and S2 (Carvell and Simons, 1986). Most of these studies exposed a restricted region of the neocortex to examine the detailed topographic organization of one or two sensory fields. The results from the current investigation support findings from these previous studies. For somatosensory cortex, neurons in S1 were responsive exclusively to tactile stimulation of the contralateral body (Nussbaumer and Van der Loos, 1985; Woolsey, 1967), and a complete representation of the contralateral body surface was described. Further, a small representation of the contralateral body surface, just lateral to S1 has been described and termed S2 (Carvell and Simons, 1986). For visual cortex, two representations of the contralateral hemifield have been described, V1 and V2 (Dräger, 1975; Wagor et al., 1980),

and are coextensive with architectonic areas 17 and 18a respectively. Neurons in these fields respond exclusively to visual stimulation. Recent studies using optical imaging techniques have identified V1 in mice (Schuett et al., 2002; Kalatsky and Stryker, 2003). These studies also identify several patches of weak activation in cortex immediately surrounding V1 and propose that these activations represent multiple extrastriate cortical fields in the mouse. However the data presented for multiple extrastriate areas are not compelling, and do not relate to the organization of extrastriate cortex determined using higher resolution electrophysiological recording techniques (e.g. Wagor et al., 1980) in which a single field, V2, was demonstrated to reside immediately lateral to V1. The issue of the organization of extrastriate cortex in rodents has been discussed in detail previously (e.g. Rosa and Krubitzer, 1999), and a detailed analysis of the data from all studies of rodents indicates that cortex immediately lateral to V1 contains only a single cortical area termed V2. Finally, it should be noted that V1 is the only darkly myelinated area in the occipital medial pole of the mouse cortex.

Studies in which the topographic organization of auditory cortex has been described (Shen et al., 1999; Stiebler et al., 1997) indicate that multiple fields are present (A1, AAF, and the ultrasonic field), that neurons in these fields respond exclusively to auditory stimulation, and that two of these fields, A1 and AAF are tonotopically organized.

Changes in cortical field organization and sensory domains

The neocortex is capable of remarkable plasticity in developing animals. Cats that sustained bilateral, high frequency, neonatal cochlear ablations developed maps in auditory cortex in which the low frequency representation had greatly expanded (Harrison et al., 1991). Further, rat pups reared in a chronically noisy environment had large disruptions in the topographic organization of A1 (Chang and Merzenich, 2003), while exposure to pure tones early in postnatal development resulted in large frequency specific expansions of the representations in A1 of the pure tone stimuli to which the neonate was exposed (Zhang et al., 2002, 2003), and a broadening of characteristic frequency for individual neurons. With very early deprivation, or loss of receptor arrays, cross-modal plasticity is even more pronounced. For example, early binocular deprivation in cats results in changes in the modality preference of neurons in the anterior ectosylvian area. Neurons that would normally respond to visual stimulation respond to auditory and somatosensory stimulation (e.g. Rauschecker and Korte, 1993). Further, these cats exhibited improved auditory localization abilities (e.g. Rauschecker, 1995). In *Monodelphis domestica* that received binocular enucleations, well before thalamocortical afferents have reached their targets, all of cortex that would have normally have been visual, contained neurons that responded to auditory and somatosensory stimulation (Kahn and Krubitzer, 2002). Given that about one third of the entire cortical sheet is devoted to processing visual inputs in *Monodelphis domestica* (e.g. Fig. 1), the plasticity observed was extreme. In addition to alterations in sensory domain allocation, the size of V1 was greatly reduced (Fig. 10C). Further, preliminary results from these studies indicate that novel cortico-cortical and thalamocortical connections had formed (Kahn et al., 2004). Particularly, area 17 had connections not only from its normal thalamic source, the lateral geniculate nucleus, but also from the ventral posterior nucleus and the medial geniculate, normally associated with the somatosensory and auditory system respectively (Fig. 10C).

As noted in the introduction, a large degree of cortical re-organization has been observed in congenitally deaf and blind humans in that cortex that would normally be involved in processing auditory and visual inputs respectively, is taken over by other sensory modalities (e.g. Catalan-Ahumada et al., 1993; Levanen et al., 1998; Weeks et al., 2000; Finney et al., 2001). Further, psychophysical studies in humans indicate that individuals who are congenitally blind are better at making sensory discriminations with the remaining sensory systems, and that the capabilities of other sensory systems can exceed that of sighted individuals. For example, using auditory event related potentials, Röder and colleagues (2000) demonstrated that there is a shorter detection time for auditory discrimination tasks in blind versus sighted individuals, and that blind individuals process language faster than sighted individuals (Röder et al., 2000). This indicates that the re-organized sensory cortex is functionally optimized, despite the lack of normal inputs.

The idea that developing sensory cortex could assume properties of another sensory system is not new. By surgically manipulating subcortical and cortical structures early in development Sur and colleagues (Pallas et al., 1990; Roe et al., 1990; Sur et al., 1999 for review) were able to induce auditory cortex to process visual inputs (Newton et al., 2004; von Melchner et al., 2000). Given this previous work, the findings in the present investigation that auditory cortex can be induced to take on properties of another sensory system are not surprising. What is intriguing about the present results is the means by which such a remarkable takeover can be accomplished. Rather than the radical surgical re-routing in which portions of cortical and subcortical structures were ablated, as was done in the studies of Sur and colleagues, the current study demonstrates that loss of sensory driven activity can have the same affect. Thus, the loss of one system may change the balance of activity between all sensory systems and dramatically alter the entire cortical sheet, including areas of the cortex that were not directly related to the sensory system in which activity was lost (e.g. V1). Further, the de-correlation between architecture and function in A1 indicates that it is possible to independently modify function and other aspects of cortical organization such as histochemical appearance and connections.

It is important to note that shifts in gene expression domains (proposed to be related to sensory domains and/or cortical fields) have been achieved via entirely different mechanisms. For example, mutant mice in which regulatory genes such as *Emx2* and *Pax6* are absent have

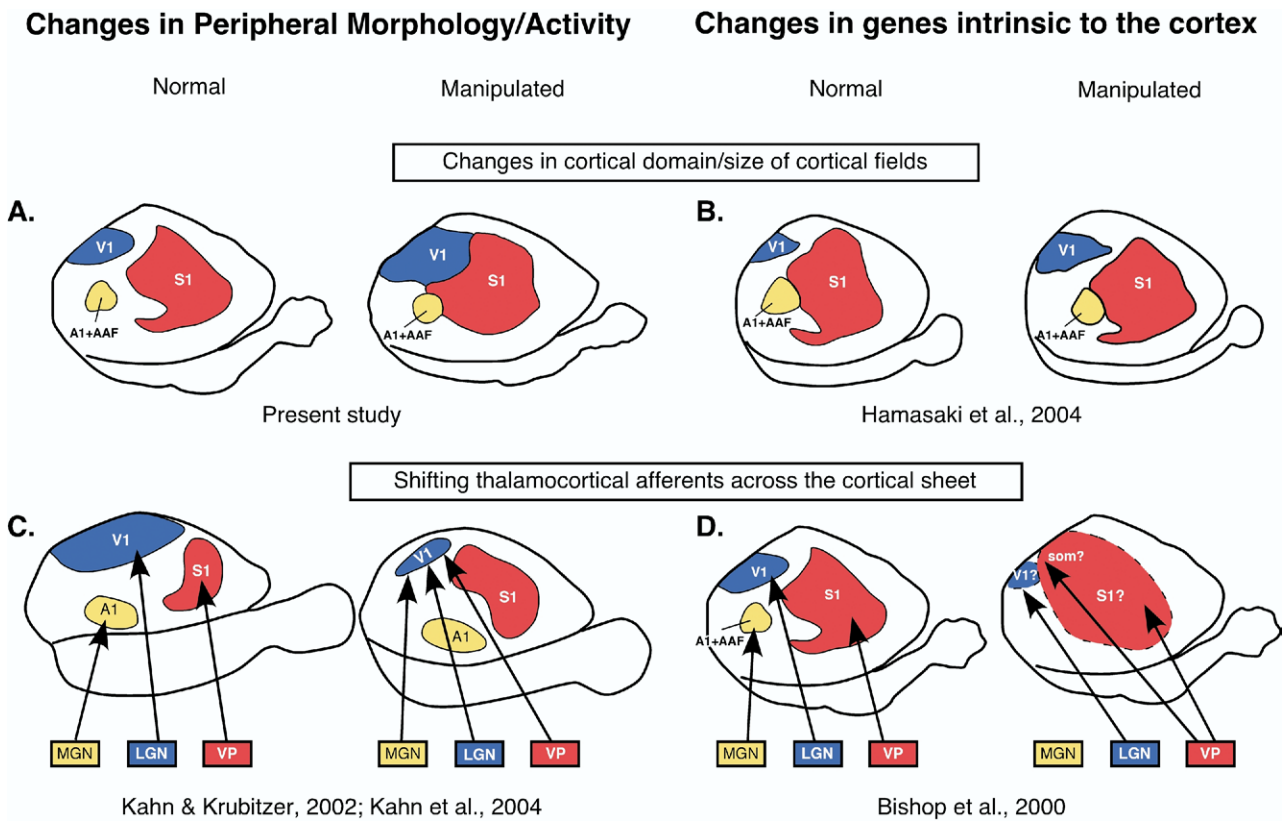


Fig. 10. A summary of results from the present investigation (A) as well as previous studies which demonstrate that similar aspects of cortical organization such as cortical field size (B), and patterns of thalamocortical connections (C and D) can be altered in the developing nervous system via different mechanisms. For example, changes in the size of a cortical field (A and B) can be accomplished by altering sensory driven activity (A), or by altering genes (overexpression of *ne-Emx2*) intrinsic to the cortex (B). Likewise, shifts in thalamocortical connections can be accomplished by altering peripheral morphology via bilateral enucleation in *Monodelphis domestica* (C), or by altering genes intrinsic to the neocortex such as the deletion of *Emx2* (D). Conventions as in previous figures.

shifts of expression of cadherins such as Cad8 and Cad6, as well as shifts in thalamocortical connectivity (Bishop et al., 2000; Fig. 10D). Alterations in patterns of gene expression have also been demonstrated for rostral regions of cortex in mutant mice in which *FgF8* is reduced (Garel et al., 2003), and cortical connections in these mutants are altered in a manner consistent with a rostral shift of cortical fields (Huffman et al., 2004). Thus, existing data indicate that both peripheral, activity-dependent mechanisms, as well as central mechanisms intrinsic to the cortex, can modify the sensory domain allocation and/or gene expression domains, cortical field functional organization, aspects of connectivity, and as described below, cortical field size (Fig. 10).

Changes in cortical field size and internal organization

While we were surprised by the extent of plasticity observed in the present investigation in a region of cortex that would normally receive auditory inputs, we were equally surprised by changes in the organization of distant fields that were not directly related to processing auditory inputs. In both of our deaf mouse models, a greater proportion of neurons in V1 responded to visual+somatosensory stimulation as compared with V1 in normal animals, and the size of V1 increased (Figs. 6, 7 and 9). As noted above, the size of area A1+AAF decreased in these animals. As with sensory domain allocation and connectivity, alterations in the size of cortical fields have been induced in developing nervous systems by either altering peripheral receptor morphology very early in developing mammals or altering genes intrinsic to the neocortex (Fig. 10A and 10B). For example, bilateral enucleations in fetal monkeys and P4 *Monodelphis domestica* resulted in a decrease in the size of area 17 (Dehay et al., 1991, 1996; Kahn and Krubitzer, 2002; Rakic et al., 1991), and neonatal enucleations in mice resulted in an increase in the size of the S1 barrel field (Bronchti et al., 1992).

On the other hand, there is evidence that signaling molecules such as *FgF8* and transcription factors such as *Emx2* regulate the size and position of cortical fields. For example, a recent study by Garel et al. (2003), in which *FgF8* has been deleted in neo/neo mice, demonstrates that such a deletion results in a rostral shift in the expression of a variety of genes involved in aspects of cortical arealization, and a concomitant reduction of the rostral molecular domain of the neocortex. In a related study, Hamasaki and colleagues (2004) genetically engineered

mice to overproduce *nestin-Emx2*. These investigators demonstrated that homozygotic mice that overexpress *Emx2* have a larger V1 than normal mice (Fig. 10B). Further, the spatial patterns of gene expression believed to be involved in aspects of arealization, such as *cad8*, were modified in a manner consistent with an expansion of V1. Taken together the data indicate that both intrinsic, genetically regulated processes, and extrinsic, activity dependent mechanisms can alter the size of a cortical field.

Potential mechanisms that subserve developmental plasticity observed in V1 and A1

We demonstrate in the current study that altering sensory driven activity from peripheral receptor arrays can alter both the size and modality specification of a cortical field. There are several potential mechanisms that could explain the functional plasticity observed in both A1+AAF and V1 in the present study. First, the reassignment of auditory cortex to the visual and somatosensory system and the observation that V1 now appears to be a bimodal rather than unimodal cortical area could be due to the unmasking of connections from multimodal cortex to A1+AAF and V1, which are normally inhibited. We do not believe this is the case since connections of at least UF in the mouse and A1 and AAF (the rostral auditory area, R) in other rodents are highly restricted to adjacent auditory regions (Hofstetter and Ehret, 1992; Luethke et al., 1988). Further, while there has never been a full description of normal connections of A1 in the mouse, preliminary studies in our laboratory indicate that, as in other rodents, connections of A1 are highly restricted to the auditory belt regions surrounding A1, and do not extend to multimodal cortex or other sensory cortex. In terms of visual cortex, cortico-cortical connections of V1 in the mouse appear to be highly restricted to areas 18a and 18b, which are unimodal visual areas that are immediately adjacent to V1 or area 17 (Simmons et al., 1982).

Another possibility is that initially exuberant cortico-cortical or thalamocortical connections in the developing neocortex fail to get pruned in deaf mice. A recent study of the development of the presumptive V1 and S1 in early postnatal mice indicates that cortico-cortical connections are highly restricted in these animals to locations immediately adjacent to the injection sites (Huffman et al., 2004). If this is indeed the case, then the exuberant connection hypothesis cannot explain the current findings. However, another investigation in which developing thalamocortical connections were described indicates that while connections are mostly modality specific, there may be some exuberance (Gurung and Fritzsch, 2004). For example, injections of lipophilic dyes into developing auditory cortex predominantly label the medial geniculate nucleus. However, examination of the data indicates that VPM/VPL and dLGN are sparsely labeled as well. Thus, a failure to prune or retract normally exuberant connections from non-auditory cortical areas and thalamic nuclei cannot be completely ruled out.

A final possibility is that there are alterations in subcortical connections in deaf mice in that nuclei that would normally be targets of auditory inputs receive visual and/or somatic inputs. We believe that such a re-routing can explain, at least in part, the current results, because re-routing at other subcortical structures, such as retinal projections to the thalamus and midbrain, is observed in deaf mice (Hunt et al., 2005). In these animals, the retina not only projects to its normal targets, such as the LGN, but also projects to non-visual targets, such as the MGN.

Taken together the present investigation and results from previous studies indicate that a number of fundamental modifications made to the mammalian neocortex can be accomplished in markedly different ways (Fig. 10). Genes intrinsic to the neocortex or alterations in the sensory receptor surface and associated patterns of activity can alter the same aspect of cortical organization, such as the size of a cortical field, in different lineages that have undergone independent evolutionary (and developmental) trajectories. In naturally evolving systems, these mechanisms likely act in concert, although not necessarily simultaneously, to generate the phenotypic variability in cortical organization observed across lineages. Thus, the neocortex of any extant mammal is a reflection of these intrinsic and extrinsic mechanisms, and is a compromise between the constraints imposed by genetically mediated developmental cascades, the physical parameters of the environment in which the animal develops, and the imposition of the morphological and behavioral adaptations selected for in any given environment.

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