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## Sound Localization Deficits During Reversible Deactivation of Primary Auditory Cortex and/or the Dorsal Zone

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<sup>1</sup>Departments of Physiology and Pharmacology, and Psychology, University of Western Ontario, London, Ontario, Canada; <sup>2</sup>School of Behavioral and Brain Sciences, University of Texas at Dallas, Richardson, Texas; <sup>3</sup>Department of Speech and Hearing Sciences, University of Washington, Seattle, Washington; and <sup>4</sup>Kresge Hearing Research Institute, University of Michigan, Ann Arbor, Michigan

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Malhotra S, Stecker GC, Middlebrooks JC, Lomber SG. Sound localization deficits during reversible deactivation of primary auditory cortex and/or the dorsal zone. J Neurophysiol 99: 1628-1642, 2008. First published January 16, 2008; doi:10.1152/jn.01228.2007. We examined the contributions of primary auditory cortex (A1) and the dorsal zone of auditory cortex (DZ) to sound localization behavior during separate and combined unilateral and bilateral deactivation. From a central visual fixation point, cats learned to make an orienting response (head movement and approach) to a 100-ms broadband noise burst emitted from a central speaker or one of 12 peripheral sites (located in front of the animal, from left 90° to right 90°, at 15° intervals) along the horizontal plane. Following training, each cat was implanted with separate cryoloops over A1 and DZ bilaterally. Unilateral deactivation of A1 or DZ or simultaneous unilateral deactivation of A1 and DZ (A1/DZ) resulted in spatial localization deficits confined to the contralateral hemifield, whereas sound localization to positions in the ipsilateral hemifield remained unaffected. Simultaneous bilateral deactivation of both A1 and DZ resulted in sound localization performance dropping from near-perfect to chance (7.7% correct) across the entire field. Errors made during bilateral deactivation of A1/DZ tended to be confined to the same hemifield as the target. However, unlike the profound sound localization deficit that occurs when A1 and DZ are deactivated together, deactivation of either A1 or DZ alone produced partial and field-specific deficits. For A1, bilateral deactivation resulted in higher error rates (performance dropping to  $\sim 45\%$ ) but relatively small errors (mostly within 30° of the target). In contrast, bilateral deactivation of DZ produced somewhat fewer errors (performance dropping to only  $\sim 60\%$  correct), but the errors tended to be larger, often into the incorrect hemifield. Therefore individual deactivation of either A1 or DZ produced specific and unique sound localization deficits. The results of the present study reveal that DZ plays a role in sound localization. Along with previous anatomical and physiological data, these behavioral data support the view that A1 and DZ are distinct cortical areas. Finally, the findings that deactivation of either A1 or DZ alone produces partial sound localization deficits, whereas deactivation of either posterior auditory field (PAF) or anterior ectosylvian sulcus (AES) produces profound sound localization deficits, suggests that PAF and AES make more significant contributions to sound localization than either A1 or DZ.

## INTRODUCTION

For many mammals, the capability to localize a sound in space and the ability to move toward or away from that sound source can be critical. Throughout the auditory pathway, multiple sites in the brain stem, midbrain, thalamus, and cortex have been identified as involved in sound localization (Jenkins and Masterton 1982; Masterton et al. 1967, 1968; Thompson and Masterton 1978). For all studied higher-order mammals (carnivores and primates) such as ferrets (Kavanagh and Kelly 1987), dogs (Girden 1939; Heffner 1978), cats (Neff 1968; Neff et al. 1956; Strominger 1969a,b; Thompson and Welker 1963), monkeys (Heffner 1997; Heffner and Heffner 1990), and humans (Neff et al. 1975; Sanchez-Longo and Forster 1958) damage of the auditory cortex produces severe sound localization deficits. Furthermore, like all other sensory cortices, the auditory cortex is not uniform, but consists of multiple areas, and each of these areas makes different contributions to sound localization (Malhotra and Lomber 2007; Malhotra et al. 2004) (Fig. 1).

In the cat, recent studies have examined the contributions of all the recognized regions of acoustically responsive auditory cortex to sound localization during both unilateral and bilateral reversible deactivation (Malhotra and Lomber 2007; Malhotra et al. 2004). These studies concluded that combined deactivations of primary auditory cortex (A1) and the dorsal zone of auditory cortex (DZ), the posterior auditory field (PAF) alone, or the auditory field of the anterior ectosylvian sulcus (FAES) alone resulted in profound sound localization deficits (combined deactivations of A1 and DZ will be indicated by "A1/ DZ"; Malhotra and Lomber 2007; Malhotra et al. 2004). Unfortunately, because these studies treated A1 and DZ together, it was impossible to discern their individual contributions to sound localization. Earlier lesion studies have also shown that A1 is critical for sound localization (Jenkins and Merzenich 1984; Masterton and Diamond 1964; Riss 1959; Strominger 1969b). However, these studies also included portions, if not all, of DZ in the ablations. Therefore no behavioral studies have examined the functional role of A1 proper, in the absence of DZ, or DZ alone.

Anatomical and physiological evidence suggests that A1 and DZ may be distinct fields of auditory cortex (He and Hashikawa 1998; He et al. 1997; Middlebrooks and Zook 1983; Stecker et al. 2005; Sutter and Schreiner 1991). DZ extends dorsally from A1 and is located on the dorsal edge of the middle ectosylvian gyrus (Fig. 1). This dorsal sector of auditory cortex was first described by Rose (1949) as the "suprasylvian fringe" (SF). Reale and Imig (1980) described the dorsal zone (DZ) of auditory cortex as the "dorsoposterior

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FIG. 1. Lateral view of the left hemisphere of cat cerebral cortex showing the positions of the 13 generally recognized areas of cat auditory cortex. Primary auditory cortex is located on the middle ectosylvian gyrus between the dorsal tips of the anterior ectosylvian sulcus and the posterior ectosylvian sulcus. The dorsal zone is shown running along the lateral lip of the suprasylvian sulcus. In subsequent figures, small versions of this schematic are used to indicate which site was deactivated. (Compiled from Clascá et al. 1997; de Ribaupierre 1997; Reale and Imig 1980; Tian and Rauschecker 1998.) A1, primary auditory cortex; AII, second auditory cortex; AAF, anterior auditory field; aes, anterior ectosylvian sulcus; dPE, dorsal posterior ectosylvian area; DZ, dorsal zone of auditory cortex; FAES, auditory field of the anterior ectosylvian sulcus; IN, insular region; iPE, intermediate posterior ectosylvian area; PAF, posterior auditory field; pes, posterior ectosylvian sulcus; ss, suprasylvian sulcus; T, temporal region; VAF, ventral auditory field; VPAF, ventral posterior auditory field; vPE, ventral posterior ectosylvian area.

area" or DP. Anatomical studies have shown that DZ may have different patterns of thalamic connections and may not be commissurally interconnected with A1 (Winer 1984). DZ has been shown to receive projections from the dorsal division of the medial geniculate (MGd) (He and Hashikawa 1998; Huang and Winer 2000; Middlebrooks and Zook 1983), whereas A1 receives its major inputs from the ventral division of the medial geniculate (MGv). A1 can also be distinguished from DZ by the presence of large pyramidal cells in layers III and V (Winguth and Winer 1986). Anatomical studies using SMI-32 (Sternberger Monoclonal, Berkeley, CA) labeling have also shown a definable border between A1 and DZ (Mellott et al. 2005). Physiological studies have revealed DZ neurons to exhibit long latencies and broad tuning curves (He and Hashikawa 1998; Middlebrooks and Zook 1983; Stecker et al. 2005), distinct from the shorter latencies and sharper tuning observed in A1, and to encode sound-source locations more accurately than A1 (Stecker et al. 2005).

Based on these anatomical and physiological differences suggesting that A1 and DZ are separate and distinct areas, the present study sought to determine the specific contributions of A1 and DZ to sound localization behavior during individual and combined deactivations. We used orienting to an acoustic stimulus as a behavioral index of sound localization and examined performance before, during, and after both unilateral and bilateral reversible cooling deactivation of A1, DZ, and both A1 and DZ (Lomber 1999). Overall, our behavioral findings support the hypothesis that DZ is a separate and independent area from A1 and that A1 and DZ each have distinct and significant roles in sound localization.

#### METHODS

Six mature (>6 mo old) domestic cats (Table 1) were obtained from a commercial laboratory animal breeding facility (Liberty Labs, Waverly, NY) and housed in a colony environment with unlimited access to water. Food intake was restricted to the behavioral training/ testing sessions and to 1 h at the conclusion of each day, when the animals had free access to dry cat food (Purina cat chow). All procedures were conducted in accordance with the Canadian Council on Animal Care's *Guide to the Care and Use of Experimental Animals*, the US National Research Council's *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (2003), and were approved by the University of Western Ontario Animal Use Subcommittee of the University Council on Animal Care and the Animal Care and Use Committee of The University of Texas at Dallas.

In each animal, after all behavioral training was complete, individual cooling loops (Lomber et al. 1999) were bilaterally implanted over A1 and DZ. After cryoloop implantation, performance on the task was tested while all cortical loci were warm (i.e., at normal physiological temperature) and active and while A1 and/or DZ was unilaterally or bilaterally cooled and deactivated.

#### Apparatus and stimuli

Training and testing were conducted in an orienting arena that allowed for the presentation of either acoustic or visual stimuli. The apparatus (Fig. 2) was a semicircular arena (diameter 90 cm) that consisted of 13 pairs of miniature speakers and red, 2-V (DC) light-emitting diodes (LEDs). The speakers (Kobitone part #25RF006, from Mouser Electronics, Mansfield, TX) were 2.5 cm in diameter with a frequency response of 800 Hz to 5 kHz. The speaker/LED combinations were mounted 15° apart along 180° of the azimuthal plane. A detailed description of the apparatus is described in an earlier publication (Malhotra and Lomber 2007). The speakers emitted broadband noise bursts (100 ms in duration). Stimuli were generated using a Tucker-Davis Technologies (Alachua, FL) stimulus presentation workstation. Stimuli were presented at 20 dB above background levels. For the stimulus, we used broadband noise bursts rather than pure tones because orienting responses to short broadband noise bursts have been identified to be much more accurate than responses to tones (Populin and Yin 1998). The apparatus was used in a dimly lit (23 cd/m<sup>2</sup>), sound-attenuated room lined in Sonex foam (Illbruck Acoustic, Minneapolis, MN).

#### Task and training

Detailed training procedures are described elsewhere (Malhotra and Lomber 2007) and will be only briefly described here. During train-

 TABLE 1. Unilaterally and bilaterally implanted loci in each of the six experimental subjects

Cat	Hemisphere	Cerebral Areas Examined	
		A1	DZ
1	L	Х	Х
	R	Х	Х
2	L	Х	Х
	R	Х	Х
3	L	Х	Х
	R	Х	Х
4	L		
	R	Х	Х
5	L		
	R	Х	Х
6	L		
	R	Х	Х

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ing, the animal oriented toward a central LED at the start of each trial. Then the LED was extinguished and the sound was presented at one of the 12 peripheral speakers or the central speaker. After the animal approached the stimulus it received a reward from the food tray below the speakers. The rapid and accurate turning of the head, or head and body, and accurate approach toward the locus of the acoustic stimulus constituted a correct orienting response. Any response other than a prompt direct approach to the appropriate stimulus was scored as incorrect. Twenty-eight trials formed a block, with each of the 12 peripheral positions tested twice and the central position tested four times. Five blocks of data were collected in each session for a total of 140 trials. Catch trials, where no target stimulus was presented, were randomly conducted. Training was complete when a criterion performance level of  $\geq$ 80% correct across the entire field was reached on two consecutive days. After the criterion was achieved, the acoustic stimuli were presented more than 100 times at each of the 12 peripheral positions examined.

As a control, the animals were also trained to orient to a visual stimulus. For the visual task, testing procedures were identical, with the only difference being that the target stimulus consisted of a flashed red 2-V (DC) LED.

## Surgical procedures

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Cooling loops were implanted after training was complete (Fig. 3). Cryoloops were fabricated by shaping loops of 23-gauge stainless steel hypodermic tubing to conform to one of the two areas examined (Lomber et al. 1999). Prior to surgery, all loops were sterilized with ethylene oxide gas. Detailed surgical procedures are described elsewhere (Lomber et al. 1999). General anesthesia was induced with sodium pentobarbital [ $\sim$ 25 mg/kg to effect, administered intravenously (iv)]. The animal was placed in a stereotaxic apparatus and craniotomies were made over the desired regions and the dura was incised and reflected to expose the cerebrum. Cryoloops were secured to the skull by using stainless steel skull screws and dental acrylic.

## Cortical loci investigated

We used reversible cooling deactivation (Lomber et al. 1999) to examine the contributions A1 and/or DZ (Fig. 3) made to acoustically mediated orienting (Fig. 1). The positions of the two loops are subsequently described.

AREA A1. An A1 loop about 7 mm long (Fig. 3) extended lengthwise across the middle ectosylvian gyrus, from the dorsal tip of the anterior



FIG. 2. Acoustic and visual orienting arena. A loudspeaker and a lightemitting diode (LED) were located above a food reward locus at each of 13 regularly spaced ( $15^\circ$ ) intervals (for sake of clarity only 30° intervals are labeled). The animal was first required to fixate on the central ( $0^\circ$ ) LED (A). It then had to orient its head to, and approach, a secondary acoustic (100-ms broadband white noise) or visual (illumination of an LED) stimulus to receive a food reward (B).



FIG. 3. Exposed left cerebrum immediately following cooling loop implantation. The cooling loops were placed below the dura and held in position with dental acrylic and skull screws. The entire A1 loop was placed in contact with the cortical surface over A1. For the DZ loop, only the lower limb of the loop was placed in contact with the cortical surface. The upper limb of the loop did not contact the brain. Left is anterior.

ectosylvian sulcus to just anterior of the posterior ectosylvian sulcus (about A2–A9; Horsley and Clarke (1908) coordinates<sup>1</sup>; Reale and Imig 1980; Figs. 1 and 3).

AREA DZ. The DZ loop was about 8 mm long (Fig. 3) and extended along the dorsal edge of the middle ectosylvian gyrus along the lip of the middle suprasylvian sulcus (about stereotaxic A2–A10; Paula-Barbosa et al. 1975; Reale and Imig 1980; Figs. 1 and 3). Only half of the lower limb of the DZ loop came in contact with the cortical surface. The upper limb did not contact the brain (Fig. 3).

As indicated in Table 1, three of the cats received unilateral placements of A1 and DZ loops, whereas three other animals received bilateral placements of A1 and DZ loops. For the bilateral placements, we implanted loops of identical shape and size in each hemisphere and stereotaxically aligned the loops in mirror-symmetric positions.

## Postsurgical procedures and implant care

Following cryoloop placement, the dura was replaced or Gelfilm (Upjohn, Kalamazoo, MI) was placed over the exposed cerebrum. With the exception of small openings where the cooling tubes exited the skull, the bone piece was replaced. Additional skull screws and acrylic were applied to secure the cooling loop and bone pieces. Dermal incisions were closed with 3–0 silk sutures that were removed 7 to 10 days later. Buprenorphine analgesic (0.01 mg/kg) was administered intramuscularly (im) during the recovery period. The cats also received Ultra-pen (Hanford Pharmaceuticals, Syracuse, NY) systemic antibiotic (300,000 units, im) for 1 wk to guard against possible infection.

## Behavioral testing and cooling deactivation

Following cooling loop implantation and prior to any deactivations, baseline performance levels were quickly reestablished. A five-step testing paradigm was used: I) baseline data were collected with all sites active; 2) testing began while a site in the left hemisphere was cooled and deactivated; 3) cooling of the homotopic site in the right hemisphere was then added to study the effects of bilateral deactivation; 4) the cooling of the left side was terminated and cortex was allowed to rewarm while the site in the right hemisphere remained deactivated; and 5) baseline levels were reestablished after cessation

<sup>&</sup>lt;sup>1</sup> Stereotaxic coordinates are provided using the Horsley and Clarke (1908) system as described by Reinoso-Suárez (1961).

of cooling and reactivation of the right side. In other sessions, this procedure was also conducted in reverse order (right, bilateral, then left) and while both A1 and DZ were simultaneously deactivated unilaterally and bilaterally. Each step in the testing paradigm consisted of at least one block of trials for cats 1–3 (see Table 1). In total, ≥24 trials were collected at each target locus both before and after cooling deactivation and  $\geq 48$  trials/locus were collected during each of the three cooling deactivation conditions. For unilateral deactivation of areas A1 and DZ, a similar five-step testing paradigm was used for cats 4-6 (see Table 1): *I*) baseline data were collected with all sites active (warm); 2) testing began while A1 was cooled and deactivated; 3) cooling of DZ was then added to study the effects of combined A1 and DZ deactivation; 4) the cooling of A1 was terminated and A1 was allowed to rewarm while DZ remained deactivated; and 5) after termination of all cooling, baseline levels were reestablished (rewarm). Each step consisted of at least one block of trials.

Cooling deactivation of cortex in contact with the cryoloop was effected by pumping cold methanol through the lumen of the cryoloop tubing. Cryoloop temperature was monitored continuously via a microthermocouple attached to the union of the loop (Lomber et al. 1999), where the tubing comes together to form the loop. Cooling of cryoloops to  $3 \pm 1^{\circ}$ C is sufficient to deactivate the full thickness cortex in contact with the cryoloop (Lomber and Payne 2000). Typical cryoloop resting temperatures range from  $36^{\circ}$ C (for gyral placements) to  $37^{\circ}$ C (for sulcal placements). During testing, the cats wore a harness and a tether that supported the cooling tubes and thermocouple wire. The harness did not restrict head or general movement of the cat. Each animal had one or two bilateral pairs of cryoloops (Table 1). The testing regime usually required 4-6 mo of daily testing.

For both orienting tasks we calculated percentage correct responses. Performance was assessed with a mixed ANOVA with one withinhemisphere variable (warm vs. cold; locus of cooling loop). Orienting responses were assessed with multifactor mixed ANOVA variables (warm vs. cold, azimuth, locus of cooling loop). The order of sessions was counterbalanced between areas (loops), functional states (active vs. deactivated), and hemispheres. When a difference was detected with the ANOVAs, we conducted follow-up within-subject t-test (Tukey test). In the RESULTS section, the P value from the t-test is provided when the difference between variables was significant. If a cooling-induced deficit was significant, additional t-tests were performed to determine whether performance was different from chance. Chance performance for the bilateral deactivations was calculated to be 7.7% correct (the task was 1/13), whereas chance performance for the unilateral deactivations was calculated to be 16.7% (the task was 1/6). The zero degree (central) position was not included in the calculations for the unilateral deactivations, only the bilateral deactivations.

## Terminal procedures

At completion of behavioral testing, one cat was anesthetized (sodium pentobarbital, 25-30 mg/kg, iv) and a small craniotomy was made, to measure temperatures beneath the cooling loops to determine the deactivated region during cooling of the cryoloops to  $3 \pm 1^{\circ}C$ (Fig. 4). The purpose of these measurements was to identify the position of the 20°C thermocline. The critical temperature below which neuronal activity is silenced is 20°C (Lomber et al. 1999). Positions between the 20°C thermocline and the cooling loop were at temperatures <20°C and were silenced, whereas positions beyond to the 20°C thermocline, relative to the cooling loop, were warmer than 20°C and partially or fully active. Cortical temperatures during cooling were measured simultaneously at four different coronal levels in the brain using multiple microthermocouples (150  $\mu$ m in diameter) manufactured for us by Omega Engineering (Stamford, CT). The microthermocouples were first positioned and then the loop was cooled to several temperatures. In all, 100 to 120 sites were sampled at each of the coronal levels. This procedure ensured that temperature

measurements for a given cryoloop temperature setting were taken at exactly the same sites in cortex. For each measurement, cortex was cooled for about 5 min prior to a recording being made, as occurred in the behavioral component of the study. This protocol was then repeated at multiple, sequentially sampled sets of sites. After completion of the mappings, the craniotomy was closed and the animal was recovered from the anesthesia using procedures described earlier.

Two days after completion of the thermal recordings and/or all behavioral studies, every cat was deeply anesthetized with sodium pentobarbital (45 mg/kg, iv) and perfused with fixatives in accordance with the recommendations of the American Veterinary Medical Association Panel on Euthanasia (Beaver et al. 2001). The brain was exposed, the position of the cryoloops was verified, and photographs were taken to provide a permanent record. The brain was cryoprotected with 30% sucrose prior to histological processing. Using a sliding microtome, the frozen brain was cut in the coronal plane. Sections were cut at a thickness of 50  $\mu$ m and stained for Nissl substance, cytochrome oxidase, or myelin.

### RESULTS

## Cortical cytoarchitecture

For each region of the auditory cortex that was cooled, the cytoarchitecture of Nissl-stained sections was characteristic of healthy cortex (Kelly and Wong 1981; Rose 1949; Sousa-Pinto 1973). We were unable to find any evidence of physical damage, gliosis, or necrosis. In addition, no changes were identified in either myelin staining or cytochrome oxidase histochemistry. Therefore in agreement with earlier studies, neither the presence of the cryoloops nor their repeated deactivation over 4-6 mo changed the structure or long-term function of the two cortical sites assayed (Yang et al. 2006).

## Unilateral and bilateral deactivations of A1 and DZ

EXTENT OF DEACTIVATIONS. Simultaneous cooling of both the A1 and DZ cryoloops resulted in deactivating the central region of the middle ectosylvian gyrus between the dorsal tips of the anterior and posterior ectosylvian sulci (Fig. 4*B*). Dorsally, this region extended to the middle suprasylvian sulcus. The deactivations were from stereotaxic A1 to A12. For each loop the deactivated region included the classically defined area A1 (Reale and Imig 1980), the dorsal zone (Middlebrooks and Zook 1983), and the region previously described as the suprasylvian fringe (Beneyto et al. 1998; Niimi and Matsuoka 1979; Paula-Barbosa et al. 1975; Woolsey 1960).

BEHAVIOR. Controls. Prior to implantation of A1 and DZ cooling loops, the three cats that received bilateral implants (Table 1) were highly proficient at the acoustic spatial localization task [90.8  $\pm$  3.1% correct across the 13 positions (mean  $\pm$  SE); Fig. 5Ai]. Following cooling loop implantation (warm), orienting response accuracy to the acoustic stimuli was virtually identical to preimplant performance levels (compare Fig. 5A, i and ii). The similarities between the pre- and postimplant performance indicate that neither the surgical implantation of the cooling loops nor the continual presence of the loops interfered with accurate sound localization. Furthermore, accurate orienting performance to every position examined returned to normal levels following the daily termination of cooling (rewarm; compare Fig. 5A, ii and iii). The similarities between the warm and rewarm performance indicate that the repeated daily cooling of the cryoloops did not



FIG. 4. Thermocline measurements taken during control conditions (A), during cooling of A1 and DZ combined (B), during cooling of A1 alone (C), and during cooling of DZ alone (D). Each quadrant contains a dorsolateral view of the cerebrum showing the positions of the A1 and DZ loops and areal extent of the deactivation shown in gray (region within the 20°C thermocline). Each quadrant also contains a coronal section through the center of the cooled cortex at approximately Horsley-Clarke level A7. The areal extent of the deactivation was determined from many coronal levels sampled in a manner similar to that shown here. For each of the 3 cooled conditions, each loop was cooled to 3°C, deactivation extended through the full thickness of cortex, and was generally limited to within 2 mm of the loop. The lumen of a loop is shown as gray to indicate when it was being cooled.

impair sound localization accuracy following rewarming of the cortex.

Deactivations. Data collected during unilateral and bilateral deactivation of A1/DZ are summarized in Fig. 5B. Unilateral cooling of the left A1/DZ significantly reduced (P < 0.01) accurate orienting responses throughout the right (contracooled) hemifield from 91.4  $\pm$  2.6% (Fig. 5Ai) to 12.8  $\pm$  4.9% correct (Fig. 5Bi). In contrast, orienting responses into the left (ipsicooled) hemifield were maintained at normally high levels  $(90.2 \pm 1.7\%$  correct before cooling;  $91.2 \pm 2.2\%$  correct during cooling) and were unaffected by the cooling deactivation. The additional cooling of right A1/DZ resulted in a deficit throughout the left hemifield that was similar in magnitude to the deficit originally identified in the right hemifield (Fig. 5Bii). In total, bilateral deactivation of A1/DZ resulted in an almost complete spatial localization impairment throughout the entire field examined, with performance significantly (P < 0.01) falling from 92.9  $\pm$  0.89% (Fig. 5Aii) to 10.7  $\pm$  2.5% correct (Fig. 5*Bii*). Performance during bilateral deactivation of A1/DZ was not different from chance (7.7%).

The termination of cooling left A1/DZ, while leaving right A1/DZ cooled, resulted in a restoration of normal orienting responses to acoustic stimuli presented in the right hemifield while continuing to leave performance in the left hemifield profoundly impaired (Fig. 5*Bi*). Compared with normal performance levels (Fig. 5*Aii*), unilateral deactivation of right A1/DZ significantly (P < 0.01) reduced orienting response accuracy in the left (contracooled) hemifield from 90.6 ± 2.5 to 13.8 ± 3.1% correct (Fig. 5*Bii*). Therefore unilateral deactivation of either the left (Fig. 5*Bi*) or right (Fig. 5*Biii*) A1/DZ resulted in mirror-symmetric sound localization deficits. Identical results were obtained when the order of cooling was reversed (right, bilateral, left) and when bilateral deactivation step.

Because there was no difference between unilateral cooling of either the left or right hemisphere, data from the unilateral



FIG. 5. Orienting responses to an acoustic stimulus during deactivation of A1 and DZ. Lateral view icons of the cat brain indicate cryoloop presence and position (gray shading), and cryoloop operational status (regions in black show that a loop was operational and that the underlying cortex was deactivated). In this and subsequent data graphs, the 2 concentric semicircles represent 50 and 100% correct response levels and the length of each bold line corresponds to the percentage of correct responses at each location tested. A: control data collected: prior to A1 and DZ cryoloop implantation (*i*), after A1 and DZ cryoloop implantation and prior to cooling in each testing session (*ii*), and shortly after termination of cooling (*iii*). B: deactivation data collected: during cooling of left A1 and DZ (*i*), during bilateral cooling of A1 and DZ (*ii*), and during cooling of right A1 and DZ (*iii*). Note that unilateral cooling of A1 and DZ reduced accurate orienting responses throughout the contracooled hemifield with no impairments in the ipsicooled hemifield. Bilateral deactivation of A1 and DZ resulted in bilateral sound localization deficits throughout the tested field.

deactivations (nine hemispheres: six from the bilaterally implanted animals and three from the unilaterally implanted animals) were pooled. Overall, unilateral deactivation of A1/DZ resulted in a decrease in response accuracy in the contralateral field falling from  $93.0 \pm 1.7$  to  $12.7 \pm 2.2\%$  correct (Fig. 6). This impaired performance was not different from chance (16.7% correct). There were no such impairment localizing targets in the ipsilateral field during unilateral deactivation. Sound localization accuracy in the ipsilateral field was  $94.5 \pm 2.8\%$  correct *before* and *after* the deactivations and  $92.5 \pm 1.9\%$  correct *during* the deactivations. The errors made during cooling deactivation will be considered in greater detail in a later section of the RESULTS (see *Sound localization errors*).

## Unilateral and bilateral deactivations of A1 alone

EXTENT OF DEACTIVATIONS. For all A1 cryoloop coolings, the central region of the middle ectosylvian gyrus between the dorsal tips of the anterior and posterior ectosylvian sulci was

deactivated (Fig. 4*C*). The deactivations were from stereotaxic A1 to A12. The deactivated region did not include the dorsalmost aspect of the middle ectosylvian gyrus, along the lateral lip of the middle suprasylvian sulcus (Fig. 4*C*). For each loop the deactivated region included the classically defined area A1 (Reale and Imig 1980).

BEHAVIOR. *Controls.* Prior to A1 cooling loop implantation, the cats were highly proficient at the acoustic spatial localization task (89.4  $\pm$  3.1% correct across the 13 positions; Fig. 7*Ai*). Following cooling loop implantation (warm), orienting response accuracy to the acoustic stimuli was virtually identical to preimplant performance levels (compare Fig. 7*A*, *i* and *ii*). Accurate orienting performance to every position examined returned to normal levels following the daily termination of cooling (rewarm; compare Fig. 7*A*, *ii* and *iii*).

*Deactivations.* Data collected during unilateral and bilateral deactivation of A1 are summarized in Fig. 7*B*. Unilateral cooling of the left A1 cryoloop significantly reduced (P <



FIG. 6. Overall accuracy during unilateral deactivation. The mean percentage correct for positions in the ipsilateral and contralateral hemifields is indicated by the height of the open bars. Height of the open bars indicates performance into the indicated hemifield during warm and rewarmed conditions combined. Height of shaded bars indicates percentage correct in the indicated hemifield during unilateral cooling of A1/DZ, A1, or DZ. Error bars indicate SE. Asterisk indicates significant difference from warm condition. Triangle indicates significant difference from A1/DZ cooling.

0.01) accurate orienting responses throughout the right (contracooled) hemifield from 88.7  $\pm$  4.3% (Fig. 7Ai) to 42.6  $\pm$ 4.9% correct (Fig. 7Biii). In contrast, orienting responses into the left (ipsicooled) hemifield were maintained at normally high levels (88.1  $\pm$  2.3% correct before cooling; 88.8  $\pm$  3.6% correct during cooling) and were unaffected by the cooling deactivation. The additional cooling of the right A1 cryoloop resulted in a deficit throughout the left hemifield that was similar in magnitude to the deficit originally identified in the right hemifield (Fig. 7*Bii*). In total, the bilateral deactivation of A1 resulted in a spatial localization impairment throughout the entire field examined, with performance significantly (P <0.01) falling from 91.1  $\pm$  1.6% (Fig. 7Aii) to 43.3  $\pm$  8.4% correct (Fig. 7Bii). However, this impairment was not nearly as large as that found during bilateral deactivation of A1/DZ (Fig. 8). In fact, the decrease in performance during bilateral A1/DZ deactivation (10.7  $\pm$  2.5% correct) was significantly greater (P < 0.01) than that identified during bilateral deactivation of A1 alone  $(43.3 \pm 8.4\% \text{ correct}; \text{ Fig. 8})$ .

The termination of cooling the left A1 cryoloop, while leaving the right A1 loop cooled, resulted in a restoration of normal orienting responses to acoustic stimuli presented in the right hemifield while continuing to leave performance in the left hemifield impaired (Fig. 7*Biii*). Compared with normal performance levels (Fig. 7*Aii*), unilateral deactivation of right A1 significantly (P < 0.01) reduced orienting response accuracy in the left (contracooled) hemifield from  $87.3 \pm 3.1$  to  $36.8 \pm 5.2\%$  (Fig. 7*Biii*). Therefore unilateral deactivation of either the left (Fig. 7*Bi*) or right (Fig. 7*Biii*) A1 resulted in mirror-symmetric sound localization deficits. Identical results were obtained when the order of cooling was reversed (right, bilateral, left) and when bilateral deactivation step.

Overall, for all six animals tested, unilateral deactivation of A1 resulted in a decrease in response accuracy in the contralateral field, falling from 90.8  $\pm$  1.9 to 45.7  $\pm$  1.1% correct (Fig. 6). This drop in performance was significant (P < 0.01). However, the impairment (45.7  $\pm$  1.1% correct) was significantly above chance (16.7% correct). In addition, there was no such impairment localizing sound sources in the ipsilateral field during unilateral deactivation of A1. Sound localization

accuracy in the ipsilateral field was  $91.5 \pm 2.3\%$  correct *before* and *after* the deactivations and  $91.1 \pm 1.5\%$  correct *during* the unilateral deactivations of A1. Therefore both unilateral (Fig. 6) and bilateral (Fig. 8) deactivations of A1 alone resulted in impairments that were not nearly as profound as those identified during similar simultaneous deactivations of A1 *and* DZ.

## Unilateral and bilateral deactivations of DZ alone

EXTENT OF DEACTIVATIONS: DZ For all DZ cryoloop coolings, the dorsal edge of the middle ectosylvian gyrus along the lip of the middle suprasylvian sulcus was deactivated (Fig. 4*D*). The region of deactivation included the dorsal-most portion of the lateral bank of the middle suprasylvian sulcus. However, the cooling did not appear to directly affect either the anterolateral (ALLS) or posterolateral (PLLS) lateral suprasylvian visual areas (Palmer et al. 1978). For each loop the deactivated region included the totality of the regions previously described as the dorsal zone (Middlebrooks and Zook 1983) and the suprasylvian fringe (Beneyto et al. 1975; Rose 1949; Woolsey 1960).

BEHAVIOR. *Controls*. Similar to control performance levels identified during cooling of A1/DZ or A1, there were no differences identified between preimplant levels (Fig. 9*Ai*) and postimplant levels either before (Fig. 9*Aii*) or after (Fig. 9*Aiii*) daily DZ cooling deactivation.

*Deactivations*. Data collected during unilateral and bilateral deactivation of DZ are summarized in Fig. 9. In the three bilaterally implanted cats, unilateral cooling of the left DZ cryoloop significantly reduced (P < 0.01) accurate orienting responses throughout the right (contracooled) hemifield from  $90.5 \pm 2.1\%$  (Fig. 9Ai) to  $73.7 \pm 3.3\%$  (Fig. 9Biv). In contrast, orienting responses into the left (ipsicooled) hemifield were maintained at normally high levels ( $91.7 \pm 1.7\%$  before cooling;  $90.9 \pm 2.5\%$  during cooling) and were unaffected by the cooling deactivation. The additional cooling of the right DZ cryoloop resulted in a deficit throughout the left hemifield that was similar in magnitude to the deficit originally identified in the right hemifield (Fig. 9*Bii*). In total, the bilateral deactivation of DZ resulted in a sound localization impairment through-



FIG. 7. Orienting responses to an acoustic stimulus during deactivation of A1. For conventions, see Fig. 5. A: control data collected: prior to A1 cryoloop implantation (*i*), after A1 cryoloop implantation and prior to cooling in each testing session (*ii*), and shortly after termination of cooling (*iii*). B: deactivation data collected: during cooling of left A1 (*i*), during bilateral cooling of A1 (*ii*), and during cooling of right A1 (*iii*).

out the entire field examined, with performance significantly (P < 0.01) falling from 92.2  $\pm$  1.3% (Fig. 9Aii) to 69.1  $\pm$ 3.8% (Fig. 9Bii). Therefore although bilateral deactivation of DZ resulted in sound localization impairments throughout the entire field, these deficits were not nearly as large as the deficits identified during bilateral deactivation of A1 alone or A1/DZ (Fig. 8). In fact, the decrease in performance during bilateral A1/DZ deactivation (10.7  $\pm$  2.5% correct) or A1 alone (43.3  $\pm$ 8.4% correct) was significantly greater (P < 0.01) than that identified during bilateral deactivation of DZ alone (69.1  $\pm$ 3.8% correct; Fig. 8). Compared with normal performance levels (Fig. 9Aii), unilateral deactivation of right DZ significantly (P < 0.01) reduced orienting response accuracy in the left (contracooled) hemifield from 90.8  $\pm$  3.7 to 72.6  $\pm$  5.5% (Fig. 9Biii). Therefore unilateral deactivation of either the left (Fig. 9Bi) or right (Fig. 9Biii) DZ resulted in mirror-symmetric sound localization deficits.

For all six animals tested, unilateral deactivation of DZ resulted in a decrease in response accuracy in the contralateral field falling from 91.7  $\pm$  2.2 to 60.2  $\pm$  2.4% correct (Fig. 6). This drop in performance was significant (P < 0.01). However, the impairment (60.2  $\pm$  2.4% correct) was significantly above chance (16.7% correct). There was no such impairment local-

izing sound sources in the ipsilateral field during unilateral deactivation. Sound localization accuracy in the ipsilateral field was 92.7  $\pm$  0.5% correct *before* and *after* the deactivations and 91.5  $\pm$  1.4% correct *during* the deactivations. Therefore both unilateral (Fig. 6) and bilateral (Fig. 8) deactivations of DZ alone resulted in impairments that were not nearly as large as those identified during similar deactivations of A1 alone or A1 and DZ together.

## Sound localization errors

During unilateral or bilateral cooling deactivations of areas A1/DZ, A1, or DZ there was a reduction in orienting response accuracy to an acoustic stimulus. Prior to cooling deactivation, the animals had previously been trained to report to the central (0°) position when they were unable to detect and localize the sound source. However, during cooling deactivation, when the animals did not respond to the correct sound location, they seldom went to the central position. Instead, we found that the animals made responses to incorrect speaker locations. Plots of the animals' responses before cooling deactivation (control) and during left, bilateral, and right deactivation of each of these three loci are provided in Fig. 10. In general, responses to the



FIG. 8. Overall accuracy during bilateral deactivation. Height of the open bars indicates combined warm and rewarm performance across all 13 tested positions. Height of the shaded bars indicates percentage correct across all positions during bilateral cooling of A1/DZ, A1 or DZ. Error bars indicate SE. Asterisk indicates significant difference from warm condition. Triangle indicates significant difference from A1/DZ cooling.

target location were highly accurate before cooling deactivation (Fig. 10*i*). During unilateral cooling deactivation of areas A1/DZ, A1, or DZ, inaccurate responses tended to occur in the correct hemifield and comprised both undershoots and overshoots (Fig. 10, *ii* and *iv*). During unilateral cooling, excellent performance was maintained in the ipsicooled hemifield. During unilateral cooling of A1 alone, sizeable numbers of errors were made to positions relatively close to the target (Fig. 10, *ii* and *iv*). In contrast, during unilateral cooling of DZ alone, fewer errors were made, but these errors tended to be further away from the target (Fig. 10, *ii* and *iv*).

Bilateral deactivation of A1/DZ, A1, or DZ yielded sound localization errors across the entire field (Fig. 10*iii*) that were consistent with the errors made during unilateral deactivation. For example, during bilateral cooling of A1/DZ, errors were made across the entire field and many errors were made to the incorrect hemifield (Fig. 10*iii*). However, when only A1 was bilaterally deactivated, the errors that were made tended to be close to the target position and the errors tended to stay in the correct hemifield (Fig. 10*iii*). On the other hand, bilateral deactivation of DZ resulted in fewer errors being made, but the errors tended to be to positions further away from the target position and many of the errors were made to positions in the incorrect hemifield (Fig. 10*iii*).

In addition to the differences in the numbers of errors made during deactivation of either A1 or DZ, we also identified differences in the magnitude of the errors made during deactivation of either A1 or DZ. During unilateral deactivation of A1 (Fig. 11A), nearly all errors were made to positions within  $30^{\circ}$  of the target. However, during unilateral cooling of DZ (Fig. 11A), most of the errors were made to positions  $\geq 60^{\circ}$ from the target. Similar results were obtained during bilateral deactivation of A1 or DZ. During bilateral deactivation of A1, nearly all errors were made to positions within  $45^{\circ}$  of the target (Fig. 11B). In contrast, during bilateral cooling of DZ, most errors were made to positions  $\geq 60^{\circ}$  from the target. Therefore although more errors were made during cooling of A1, the errors that were made during cooling of DZ tended to be much larger.

The larger magnitude of errors during either unilateral or bilateral deactivation of DZ versus A1 included a greater percentage of targeting errors into the incorrect hemifield. The percentage of errors made to the incorrect hemifield during unilateral and bilateral deactivations of A1/DZ, A1, and DZ are summarized in Fig. 12. Specifically, during bilateral deactivation of DZ,  $26.9 \pm 5.2\%$  of the errors were made to the incorrect hemifield (Fig. 12B). In contrast, during bilateral deactivation of A1,  $3.7 \pm 1.8\%$  of the errors were to the incorrect hemifield (Fig. 12B). A similar, but not as large, difference was identified during unilateral deactivation of DZ (errors,  $15.9 \pm 3.5$ ) or A1 (errors,  $5.8 \pm 2.2$ ; Fig. 12A). Therefore although more errors were made during cooling of A1, the errors that were made during cooling of DZ tended to be much larger and were more likely to be made to the incorrect hemifield.

## Orienting to visual stimuli

To confirm that the acoustic spatial localization errors we identified during cooling of A1/DZ, A1, or DZ were unique to one modality and not general motor deficits, we also examined the ability of the cats' to orient to a visual stimulus introduced at the exact same spatial locations examined with the acoustic stimuli. In this paradigm, the testing procedures were identical, with the only difference being that the 100-ms broadband acoustic stimulus (target stimulus) was replaced with a flashed red 2-V (DC) LED. During unilateral or bilateral cooling deactivation of A1/DZ, A1, or DZ, no significant changes in orienting to visual targets were identified anywhere in the visual field. This result suggests that the acoustic spatial localization deficits identified during unilateral and bilateral deactivations of A1/DZ, A1, or DZ were specific acoustic impairments without accompanying visual or motor deficits.

## DISCUSSION

In our earlier studies, we examined the contributions of numerous cortical regions, both within and outside auditory cortex, to accurate orienting toward an acoustic stimulus (Malhotra and Lomber 2007; Malhotra et al. 2004). In those investigations we treated A1 and DZ as one cortical area and deactivated both regions simultaneously with one cooling loop. The findings revealed that unilateral deactivation of A1/DZ resulted in a profound reduction in accurate orienting to targets in the contralateral, but not ipsilateral, hemifield (Malhotra et al. 2004). Furthermore, bilateral deactivation of A1/DZ reduced accuracy to chance levels and resulted in deficits across the entire field (Malhotra and Lomber 2007). Errors made during bilateral deactivation of A1/DZ tended to be confined to the same hemifield as the target (Malhotra and Lomber 2007). These results were confirmed in the present study.

In this study, we examined the behavioral contributions of A1 and DZ to sound localization during separate and combined unilateral and bilateral deactivations. For both areas, unilateral deactivation resulted in sound localization deficits restricted to the hemifield contralateral to the deactivation. Bilateral deactivation of these two areas resulted in sound localization deficits throughout the entire field. However, unlike the pro-



FIG. 9. Orienting responses to an acoustic stimulus during deactivation of DZ. For conventions, see Fig. 5. A: control data collected: prior to DZ cryoloop implantation (*i*), after DZ cryoloop implantation and prior to cooling in each testing session (*ii*), and shortly after termination of cooling (*iii*). B: deactivation data collected: during cooling of left DZ (*i*), during bilateral cooling of DZ (*ii*), and during cooling of right DZ (*iii*).

found sound localization deficit that occurs when A1/DZ are deactivated together (present study; Malhotra and Lomber 2007; Malhotra et al. 2004), deactivation of either A1 or DZ alone produced partial and distinct deficits. For A1, bilateral deactivation resulted in sound localization performance dropping from about 90 to 45% correct. The errors made during the A1 deactivations tended to be within  $\leq 30^{\circ}$  of the target and were almost always made to the same hemifield as the target. In contrast, with bilateral deactivation of DZ, sound localization performance dropped from about 90 to 60% correct. The errors made during the DZ deactivations tended to be  $\geq 60^{\circ}$ from the target and large numbers of errors were made to the incorrect hemifield. Therefore individual deactivation of either A1 or DZ produced specific and unique sound localization deficits. The results of the present study suggest that: 1) DZ plays a role in sound localization; 2) DZ and A1 are distinct cortical areas; and that 3) the contributions of other cortical regions (PAF and FAES) to sound localization may be more significant than either A1 or DZ.

## Are AI and DZ distinct auditory areas?

To determine whether two cortical areas should be designated as distinct from one another, particularly when the areas are adjacent to each another, it is critical to evaluate the multiple criteria that need to be considered to differentiate two cortical regions. Rosenquist (1985) described that cortical areas can be differentiated in five ways: 1) cyto- or myeloarchitectonic, or histochemical differences; 2) differences in cortical connections; 3) topographic or mapping criteria; 4) differences in the receptive field properties of the neurons; and 5) differences in behavior following stimulation or inactivation. Although many regions of the cortex are deemed different from one another on all the preceding criteria, for some areas we must make a decision based on a preponderance of the evidence. However, in the case of A1 and DZ, it seems that these two areas can now be distinguished on the basis of all five criteria.

DIFFERENCES IN CYTO- OR MYELOARCHITECTURE, OR HISTOCHEMIS-TRY. Although the differences are subtle, the border between A1 and DZ can be identified in Nissl- and myelin-stained tissue (Winer 1984, 1985; Winguth and Winer 1986). Specifically, the A1/DZ border can be recognized by the presence of many large pyramidal cells in layers III and V. Compared with A1, DZ has a less granular appearance, distinctly darker myeloarchitecture, and a less well differentiated layer III (Winer 1984, 1985; Winguth and Winer 1986). More recently, SMI-32 has



FIG. 10. Spatial localization accuracy to the acoustic target. Target position is indicated on the *x*-axis (negative values indicate left hemifield). Orienting response is indicated on the *y*-axis. For each target location, the area of each circle indicates the percentage of responses to a given speaker position. *i*: accuracy before and after any cooling deactivations. *ii*: accuracy during left cooling deactivation. Note errors in right hemifield. *iii*: accuracy during bilateral deactivation. Note errors in left hemifield.

been used to determine a definable border between A1 and DZ (Mellott et al. 2005). SMI-32 is a monoclonal antibody that recognizes a nonphosphorylated epitope on the medium- and high-molecular-weight subunits of neurofilament proteins (Sternberger and Sternberger 1983). The antibody labels primarily the somata and dendrites of pyramidal neurons in cortical layers III, V, and VI and has been extensively used to parse visual cortex in the monkey and cat (Hof and Morrison 1995; Lewis and Van Essen 2000; Van der Gucht et al. 2001). In SMI-32–reacted tissue, both A1 and DZ contain large labeled somata in lower layer III and upper layer V, and labeling in the infragranular layers appears identical. However, in the supragranular layers of DZ, dendritic labeling in layer III is significantly denser than in A1 (Mellott et al. 2005). This difference permits the border between

A1 and DZ to be readily identified, even at low-power magnification (Mellott et al. 2005). Therefore areas DZ and A1 can be distinguished from one another based on cytoarchitectonic, myeloarchitectonic, and histochemical differences.

DIFFERENCES IN CORTICAL CONNECTIONS. DZ and A1 may also be distinguished by their unique patterns of thalamic and cortical inputs. With regard to thalamocortical projections, DZ is innervated by cells in the dorsal division of the medial geniculate body (dorsal and deep dorsal nuclei; He and Hashikawa 1998; Huang and Winer 2000; Winer and Lee 2007) and from the dorsal cap of the ventral division (Middlebrooks and Zook 1983), whereas A1 is dominated by innervation from the ventral division of the medial geniculate body (Huang and



FIG. 11. Cumulative percentage responses as a function of magnitude of error during (*A*) unilateral and (*B*) bilateral deactivation of A1/DZ (black), A1 (light grey), or DZ (dark grey). *A*: responses are shown for the contracooled hemifield. *B*: responses shown for all 13 tested positions across both hemifelds. Note that for A1/DZ and A1 deactivations that most errors are  $\leq 30^{\circ}$  of the target, whereas for DZ deactivations that most errors are  $\geq 45^{\circ}$  from the target.

Winer 2000; Winer and Lee 2007). From the ipsilateral auditory cortex, DZ receives its densest cortical afferents from A1 and PAF, with moderate projections arising from second auditory cortex (AII) and the anterior auditory field (AAF; He and Hashikawa 1998; Winer and Lee 2007). In contrast, A1 receives dense cortical afferents from AAF, PAF, and the ventral posterior auditory field (VPAF), with moderate projections arising from the ventral auditory field (VAF), DZ, and AII (Winer and Lee 2007). Finally, DZ receives only sparse or moderate projections from the contralateral hemisphere, whereas A1 has very dense transcallosal projections with its homotopic counterpart in the opposite hemisphere. Therefore areas DZ and A1 can be distinguished from one another based on patterns of connections within the telencephalon.

DIFFERENCES IN TOPOGRAPHIC OR MAPPING CRITERIA. Primary auditory cortex was the first region of acoustically responsive cortex to be mapped according to the distribution of characteristic frequencies (CFs; Merzenich et al. 1975; Reale and Imig 1980). In A1, neurons are tonotopically organized with the highest CFs (35–40 kHz) located rostrally on the middle ectosylvian gyrus and neurons with the lowest CFs (0.1-1 kHz) located caudoventrally on the anterior bank of the posterior ectosylvian sulcus (Merzenich et al. 1975; Reale and Imig 1980). A decreasing progression of CFs is encountered along a path extending from the highest to the lowest portions of the frequency representation. The positions of A1 neurons with similar CFs define isofrequency bands that are oriented orthogonally to the low-to-high CF gradient. Therefore on the middle ectosylvian gyrus these bands are generally oriented in a dorsoventral direction (Merzenich et al. 1975; Reale and Imig 1980). However, within A1, not all frequencies are represented equally because the upper three octaves occupy a proportionally larger region of cortex than the lower two (Merzenich et al. 1975). In addition to A1, areas AAF (Knight 1977; Phillips and Irvine 1982; Reale and Imig 1980), PAF (Phillips and Orman 1984; Reale and Imig 1980), and VPAF (Reale and Imig 1980) have also been identified to have a tonotopic organization. In contrast, area DZ does not contain an orderly tonotopic map similar to that identified in A1. Earlier studies reported that neurons in area DZ could not be assigned a CF or were poorly driven by tone bursts regardless of intensity (Merzenich et al. 1975). Reale and Imig (1980) described that the CFs of neurons in DZ were not consistent with the tonotopic organization of A1. Finally, Middlebrooks and Zook (1983) found that frequency tuning of DZ units tended to be broader and shifted to higher frequencies than in corresponding anteriorposterior locations in A1. Therefore A1 and DZ can be distinguished from each other based on the presence or absence of tonotopic organization, respectively.

DIFFERENCES IN RECEPTIVE FIELD PROPERTIES. Electrophysiological studies of the neuronal response properties of neurons in A1 and DZ have revealed significant differences between neurons in A1 and DZ. In comparison to A1, DZ neurons have longer-latency responses and tuning curves that are broad and/or multipeaked (He and Hashikawa 1998; He et al. 1997; Middlebrooks and Zook 1983; Sutter and Schreiner 1991). Compared with A1, neurons in DZ have more complex frequency tuning and increased prevalence and degree of nonmonotonic rate-level functions (Stecker et al. 2005). With regard to spatial sensitivity, DZ neurons have sharper spatial tuning and their response latencies are more strongly modulated by stimulus location than are neurons in A1 (Stecker et al. 2005). Sutter and Schreiner (1991) described that, compared with A1, neurons in DZ often provide better responses to white noise than to tones and that DZ neurons were often difficult to drive with monaural contralateral tones. Finally, neurons in DZ have been identified to have excitatory-excitatory binaural response properties, whereas excitatory-excitatory binaural neurons in A1 are segregated from excitatory-inhibitory neurons in alternating bands that run anteroposteriorly across A1 (Imig and Adrián 1977; Middlebrooks and Zook 1983). Although earlier physiological reports had considered DZ to be part of A1 (Andersen et al. 1980; Middlebrooks and Zook 1983; Woolsey 1964), more recent detailed investigations (He and Hashikawa 1998; Stecker et al. 2005) have provided ample evidence that A1 and DZ can be differentiated on the basis of neuronal receptive field properties.

DIFFERENCES IN BEHAVIOR FOLLOWING STIMULATION OR INACTIVA-TION. The present report is the only study to directly compare the affects of deactivating A1 or DZ on any acoustic behavior.





FIG. 12. Percentage of total errors made to the incorrect hemifield during (A) unilateral or (B) bilateral deactivation of A1/DZ, A1, or DZ. Error bars indicate SE.

The results from the present study show that bilateral deactivation of A1 produces a moderate sound localization impairment with nearly all errors occurring in the same hemifield as the target and most errors occurring  $\leq 30^{\circ}$  from the target. In contrast, bilateral deactivation of DZ results in fewer sound localization errors. However, the errors that do occur are generally quite large ( $\geq 45^{\circ}$ ) and are often to the incorrect hemifield. Therefore A1 and DZ can be distinguished from each other based on differences in behavior following inactivation.

The combined deactivations of A1 and DZ produce profound acoustic orienting deficits that reduce performance to chance levels (present study; Malhotra and Lomber 2007). However, independent deactivations of A1 or DZ result in only partial decreases in sound localization behavior. Therefore it could be argued that A1 and DZ may constitute a single cortical region that plays a major role in sound localization similar to PAF and FAES (Malhotra and Lomber 2007; Malhotra et al. 2004). However, this proposition requires the expectation that if a cortical region is involved in sound localization that deactivating the region must result in a robust and severe deficit. This expectation is flawed and it is likely that several regions in cortex work in concert to produce sound localization behaviors (Hall 2003; Middlebrooks 2002; Middlebrooks et al. 2002; Zatorre et al. 2002). Overall, A1 and DZ can be differentiated from one another on each of the five criteria described by Rosenquist (1985). These findings buttress the idea that DZ is a separate and independent area from A1 and it plays an important role in sound localization.

## Are A1 and DZ portions of a larger area?

The present behavioral study supports the proposal that A1 and DZ are separate cortical areas. An alternative explanation is that the A1 and DZ cooling loops inactivated distinct portions of a single topographic map. We do not favor the latter explanation because it would require a topographical map in A1, which is inconsistent with the large body of physiological data available on A1 (e.g., Imig et al. 1990; Middlebrooks and Pettigrew 1981: Middlebrooks et al. 1998: Rajan et al. 1990). In addition, there has been some speculation that "spatiotopic bands" might exist in A1, lying orthogonal to the isofrequency bands, but no such bands have been demonstrated. Furthermore, "binaural bands" present in A1 (Imig and Adrián 1977; Middlebrooks and Zook 1983; Middlebrooks et al. 1980) might predict an alternation between frontal and lateral spatial sensitivity within a hemifield, but not a topographic organization across the dorsoventral dimension of A1. It may be that, in the present experiment, cooling of various regions involved varying balances of subregions that process excitatory-excitatory

(EE)/excitatory-inhibitory (EI) or left/right across both fields (A1 and DZ), and that the partial deficits reflect the particular balance. This interpretation would predict that cooling central A1 or DZ should have produced localization errors or response bias within particular spatial regions (specifically, greater errors in frontal areas with DZ cooling). In contrast, the results show that deactivation of DZ tended to produce infrequent large errors, with no spatial pattern. Finally, without a principled model of how the binaural bands interact to subserve sound localization, we cannot predict how partial deactivation of those bands should affect behavior.

# Not all cortical regions contribute to sound localization equally

Our recent studies have shown that unilateral or bilateral deactivations of A1/DZ, PAF, or FAES resulted in profound sound localization deficits (Malhotra and Lomber 2007; Malhotra et al. 2004). However, these studies also revealed that neither unilateral nor bilateral deactivation of AAF, VPAF, AII, insular region (IN), temporal region (T), VAF, dorsal posterior ectosylvian area (dPE), intermediate posterior ectosylvian area (vPE) had any effect on the sound localization task (Malhotra and Lomber 2007). Therefore one major conclusion that could be drawn from these earlier results is that most of auditory cortex is not necessary for accurate sound localization.

Other conclusions can also be drawn when the results from this present study are compared with earlier studies. First, although A1 does play a role in sound localization, its role is not as significant as that described in earlier reports. The earliest reports implicating A1 in sound localization involved large physical ablations of A1 and much or all of the remaining acoustically responsive cortex (Neff 1968; Neff et al. 1956; Strominger 1969a,b; Thompson and Welker 1963). These studies reported significant sound localization deficits following large lesions in auditory cortex. Subsequent studies made smaller lesions that included A1 (Jenkins and Merzenich 1984; Masterton and Diamond 1964; Riss 1959; Strominger 1969b). However, these studies also included portions, if not all, of DZ in their ablations. Even the most recent reversible deactivation studies examining the contributions of A1 did not investigate the contributions of A1 alone, but examined the contributions of A1 together with DZ (Malhotra and Lomber 2007; Malhotra et al. 2004). Both the later ablations studies and reversible deactivation experiments described profound sound localization deficits following lesion or inactivation of A1/DZ. Therefore it was impossible to discern the individual contributions of A1 or DZ from any previous studies. In the present study we explicitly examined the individual contributions of both A1 and DZ to sound localization behavior. The present results show that deactivations restricted to A1 alone do not produce deficits that are as severe as those reported by earlier studies (Jenkins and Merzenich 1984; Malhotra and Lomber 2007; Malhotra et al. 2004; Masterton and Diamond 1964; Riss 1959; Strominger 1969b). Therefore although primary auditory cortex does play a role in sound localization, its role may not be as significant as that described in earlier reports.

Second, PAF and FAES each play more critical roles in coordinating accurate orienting to an acoustic stimulus that either A1 or DZ. Earlier studies have reported that deactivation of A1/DZ, PAF, or FAES results in sound localization deficits that reduce normal performance to chance levels (Malhotra and Lomber 2007; Malhotra et al. 2004). The present study examined A1 and DZ individually and revealed that deactivation of neither area results in deficits as severe as those identified during deactivation of PAF or FAES (Malhotra and Lomber 2007; Malhotra et al. 2004). Therefore the roles of PAF and FAES in sound localization appear to be more significant that either A1 or DZ. Considering the positions of FAES and PAF in a proposed sound localization pathway in auditory cortex (Lomber et al. 2007) we hypothesize that PAF is more involved in the perceptual machinery underlying sound localization of sound localization behaviors.

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