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BRIEF COMMUNICATION

# A Deoxyglucose Study on Auditory Responses in the Bat *Rhinolophus rouxi*

PETER MELZER[1]

*AK NRP, Zoologisches Institut der J. W. Goethe Universität*
*Siesmayerstr 70, D-6000 Frankfurt am Main, FRG*
*and Institute of Anatomy, University of Lausanne, Rue du Bugnon 9, 1005 Lausanne, Switzerland*

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MELZER, P. *A deoxyglucose study on auditory responses in the bat* Rhinolophus rouxi. BRAIN RES BULL15(6) 677–681, 1985.—Responses in the auditory system of the echolocating bat *Rhinolophus rouxi* were mapped with [$^3$H]deoxyglucose (DG) autoradiography. After unilateral stimulation with the constant frequency component of the individual bat's echolocation call, stimulus-related DG uptake occurred contralaterally in the dorsolateral and the ventromedial parts of the inferior colliculus, in the ventral medial geniculate body and in the contralateral neocortex. Unilateral 50 kHz-stimulation gave rise to a weakly activated lamina in the dorsolateral contralateral inferior colliculus. The distribution of frequency-evoked DG uptake within the inferior colliculus agrees with electrophysiological findings on an acoustic fovea in the auditory pathway of echolocating bats.

Deoxyglucose autoradiography Auditory pathway tonotopy *Rhinolophus rouxi*

LIKE the greater horseshoe bat, *Rhinolophus ferrumequinum*, the rufous horseshoe bat, *Rhinolophus rouxi*, from southern India emits echolocation calls composed of a constant frequency *(CF)* component between 70 and 85 kHz (depending on the individual) and a modulated frequency *(FM)* component. If the bat approaches a close target, the frequencies of the echoes to its echolocation calls are significantly increased owing to the Doppler-effect. Yet, the echoes of the *FM* component remain close to the bat's *CF* component [10]. The bat's highest frequency resolution is in the range of the CF component, and its auditory system displays corresponding structural and physiological adaptations both peripherally [2] and centrally [12,13]. Deoxyglucose (DG) autoradiography [19] was used to demonstrate the central representation of neuron clusters responding to the bat's *CF* or to a lower frequency (50 kHz) for comparison. The aim of this approach was to correlate the spatial distribution of stimulus-related DG uptake in the inferior colliculus with the tonotopical organization as deduced from single- and multi-unit recordings [12]. The visualization of the complete spatial extent of frequency-specific neuronal activity was thought to substantiate electrophysiological findings.

## METHOD

Four male adult animals, weighing 12.5–16.5 g, were deprived of food for 24 hours and anesthetized with halothane (3%) prior to stimulation. In a sound-attenuated chamber, the animals were stimulated in their right ears (their left ears were occluded with histoacrylate cement) with tone bursts (pulse duration 80 msec; rise and fall time 1 msec; repetition rate 6.25/sec; intensity 45 dB SPL). For one animal, the tone frequency was 50 kHz; two others were stimulated with their individual *CFs* (84.4 and 85.5 kHz) determined with a measuring amplifier (Brüel and Kjaer, model 2610). Immediately after the onset of stimulation, 2-[1,2-$^3$H]-deoxy-D-glucose (New England Nuclear) in saline (500 $\mu$Ci/100 g b.w.) was injected intraperitoneally. The ambient temperature was maintained at 26°C. After 30 minutes, the animals were killed by a halothane overdose; their brains were removed and immediately frozen at -70°C. They were then mounted onto object holders with chilled No. M-1 embedding matrix (Lipshaw Manufacturing Co.). Twenty micron thick coronal sections were processed following the conventional method [18]. As a control, one bat underwent the whole procedure without tone burst stimulation. Autoradiograms on Ultrofilm $^3$H (LKB) were obtained as described elsewhere [9]. The sections were stained with cresylecht violet. Regions on autoradiograms were specified by superimposing the image of the corresponding counterstained section with an optical comparison bridge (Zeiss). Autoradiographic optical densities (O.D.s), as indicators of DG uptake, were measured with a densitometer as described elsewhere [9]. For all measurements, the O.D. given by the background of the autoradiographic film was subtracted.

[1]Requests for reprints should be addressed to P. Melzer, Institute of Anatomy, University of Lausanne, Rue du Bugnon 9, CH-1005 Lausanne, Switzerland.

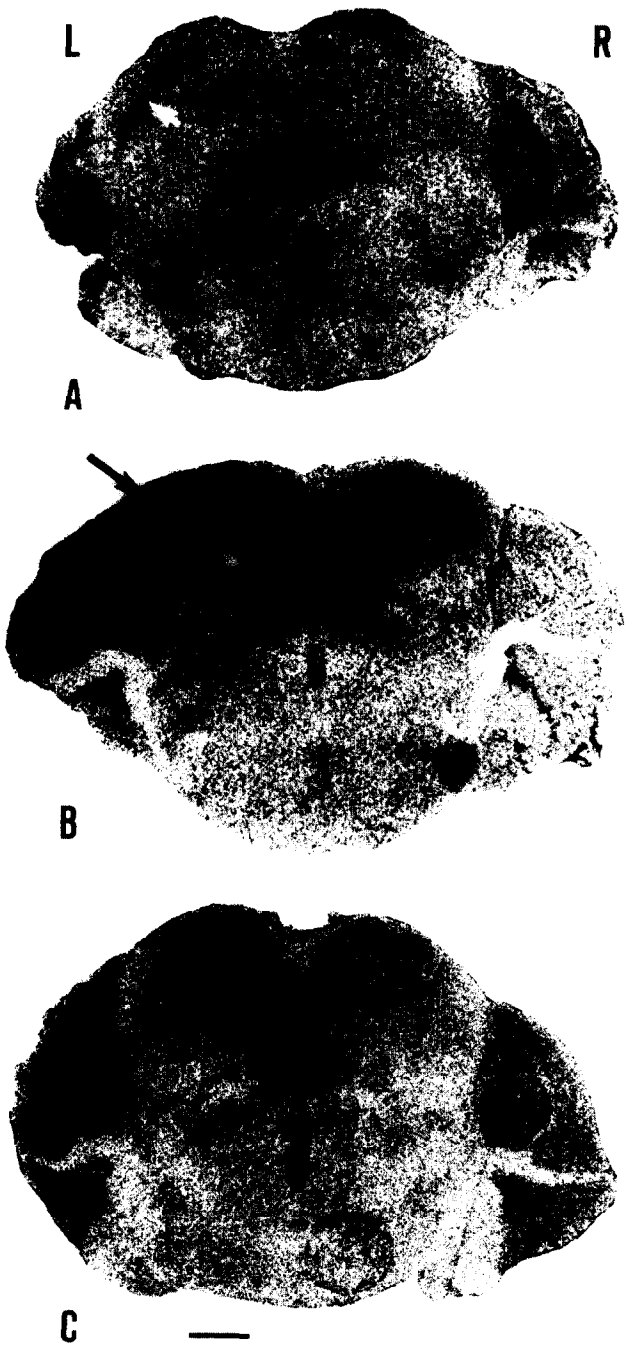


FIG. 1. Autoradiograms of transverse sections of animals stimulated on the right side with 50 kHz (A), with the individual *CF* (B) and a non-stimulated control (C). The animals were anesthetized with halothane. The sections are from the equivalent midbrain regions. Stimulus-related DG uptake (black and white arrows) is seen only contralateral to the sound source. In B, the lamina of high DG uptake in ICC/vm (white arrow) agrees with electrophysiological findings, but the dorsolateral zone of high DG uptake (black arrow) has not yet been described electrophysiologically. It was present in both the *CF*-stimulated animals. None of the effects obvious in A and B occurred in the control (C). The bar in C, bottom, indicates 1 mm. Dorsal is up; the animal's right side is right (in A: L—left, R—right).

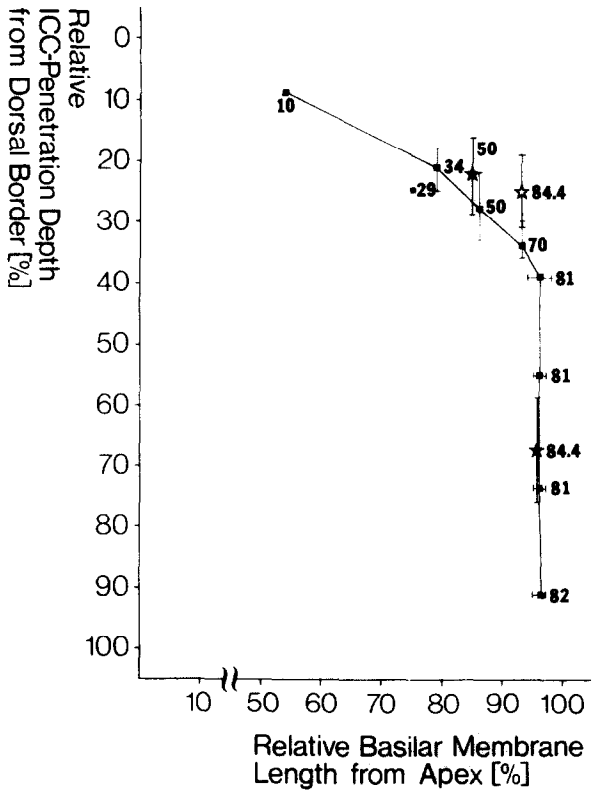


FIG. 2. Plot of relative ICC penetration depth (as percentage of the penetration depth within which acoustic neurons were recorded) for a given *BF* as a function of distance (as percentage of total basilar membrane length) from the apex to the point that represents the same frequency on the cochlea. Values for penetration depths and corresponding *BFs* for *Rhinolophus ferrumequinum* (squares) were obtained by averaging data published by Pollak and Schuller [12]. The ordinate values for *Rhinolophus rouxi* (asterisks) were derived from averaged ICC area measurements on autoradiograms from one animal for each frequency. Note the agreement of the tonotopy recorded electrophysiologically and the laminae of high DG uptake indicated by white arrows in Fig. 1 (filled asterisks). The open asterisk indicates the dorsolateral region of high DG uptake evoked by 84.4 kHz (Fig. 1B; black arrow). It is close to the averaged penetration depth, at which neuronal *BFs* of 70 kHz were obtained. Seventy kHz is considered to be the highest frequency encountered within the ICC/dl [12]. Thus, the corresponding areas of high DG uptake cover most of ICC/dl. Data for the relative basilar membrane length corresponding to a certain frequency was calculated from a conversion function developed by Greenwood [6]. The basilar membrane lengths used were 16.1 mm for *Rhinolophus ferrumequinum* and 15.6 mm for *Rhinolophus rouxi* respectively.

From each autoradiogram at least three O.D. scans were obtained in areas of interest; their mean O.D. was determined. In addition, pseudocolor coded images of selected autoradiograms were produced with a camera densitometer (Max-Planck-Institut für Hirnforschung, Abt. Prof. W. Singer, Frankfurt a.M., FRG).

## RESULTS

Only weak sound-specific DG uptake could be observed in the cochlear nucleus and superior olivary complex. High tracer accumulation was evoked in the nuclei of the lateral lemniscus bilaterally, but this was not frequency-specific. The most obvious response occurred in the inferior col-

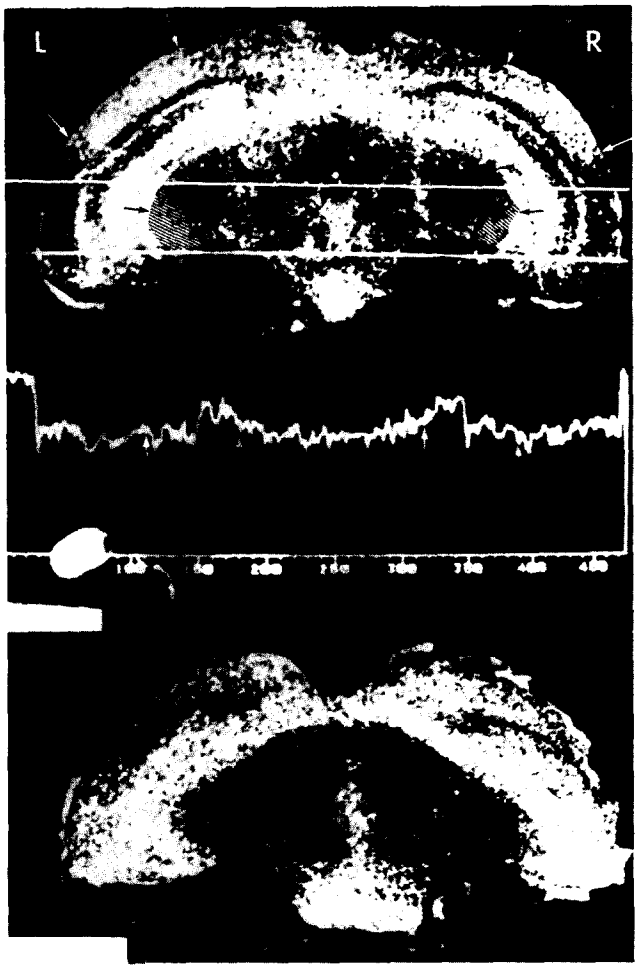


FIG. 3. Black and white reproductions of pseudocolor camera densitometer images of autoradiograms of coronal sections from matching diencephalic levels (top: bat stimulated with its *CF* on the right; bottom: non-stimulated animal). The images were composed of optical density measurements within a "window" of the spectrum of gray. The window's lower threshold was shown as black, its upper threshold as white. Ten colors were interpolated, increasing from blue to red, each representing a limited range of optical densities. Due to the photographic processing all colors but blue turned almost white (see color scales at the left bottom corners of the images). Thus, it is still to discriminate at least three gray levels. At the bottom of the top image, transmission readings across the image are displayed (Abscissa: distance, 1 unit equals 100 $\mu$m; ordinate: arbitrary units). They cover the area between the horizontal white lines. Each value on the ordinate represents the mean transmission of all pixels in a vertical column between the lines. The measurements covering the left and the right MGB are between the white arrows. They indicate a slightly higher DG uptake in the left MGB which is contralateral to the stimulus. Furthermore, the area of highest DG uptake is larger on the contralateral than on the ipsilateral side (shaded areas; black arrows). Both cortical hemispheres show increased DG uptake in restricted areas (between white arrows). Contralateral to stimulation (left), the overall uptake was slightly higher than on the ipsilateral side (right). The effects could be traced on consecutive autoradiograms; none was found in the non-stimulated animal. Orientation: dorsal is up; the animal's right side is right (at the top: L—left, R—right).

liculus (IC) contralateral to the sound source (Fig. 1). At stimulation with the individual *CF*, there was a region of high DG uptake dorsally and laterally in the IC's central nucleus (ICC; Fig. 1B, black arrow). It spanned ca. 1100 $\mu$m rostrocaudally. Area measurements on consecutive autoradiograms (Morphomat 10, Zeiss) showed that it occupied 24.5% (n=11; S.D.=6.0%) of the total area of the ICC. In the coronal plane, it seemed to be composed of three adjacent bands. Compared with the Nissl-stained sections, they covered most of the *dorsolateral region of the ICC* (ICC/dl) described by Schweizer [15]. A second region of high DG uptake (Fig. 1B; white arrow) forming a layer, 150 $\mu$m thick and 700 $\mu$m long (rostrocaudally), was found at the dorsoventral center of the *ventromedial region of the ICC* (ICC/vm). Its O.D. was 1.8-fold higher than that of adjacent tissue. The mean area dorsal to the middle of the layer covered 67.0% (n=8; S.D.=8.5%) of the ICC on consecutive autoradiograms. The 50 kHz-stimulus gave rise to only a single lamina of slightly-enhanced DG uptake within the ICC/dl (Fig. 1A; white arrow). Its optical density was 1.2 times that of the surrounding tissue. The dorsal ICC area partition was 22.1% (n=14; S.D.=6.2%).

The mean proportions of areas dorsal to the laminae of frequency-evoked DG uptake were used as measures of the relative depths of these laminae within the ICC. The results of Servière *et al.* [17] imply that the mean area proportion for a given frequency must equal the average relative penetration depth at which the same frequency was recorded from single- and multi-units at the lowest sound intensity, i.e., as the units' *best frequency (BF)*. Relative penetration depths are expressed as percentages of the total penetration depths within which acoustic responses occurred. For the comparison of the depths of frequency-specific laminae within the ICC derived from this study with the tonotopy of the ICC known from electrophysiological recordings in *Rhinolophus ferrumequinum* [12], see Fig. 2. As in other mammals [1], ICC/dl and ICC/vm in rhinolophides form a single tonotopical unit with a progression from low *BFs* in the dorsolateral to high *BFs* in the ventromedial aspect of the ICC [15]. Since the basilar membrane length of *Rhinolophus rouxi* is ca. 15.6 mm (personal communication by M. Vater; see also [3]) and that of *Rhinolophus ferrumequinum* is 16.1 mm [2], the locus of a given frequency's representation on the basilar membrane of the cochlea, expressed as relative distance from the apex, has been calculated using $x=7.67 \log_{10}(1.28\times10^{-3}f+1)$ for *Rhinolophus ferrumequinum* and $x=7.42 \log_{10}(1.20\times10^{-3}f+1)$ for *Rhinolophus rouxi* where $x$ represents distance from apex in mm and $f$ frequency in Hz. The two formulae were derived from a basilar membrane locus-frequency function determined in psychoacoustic experiments on man and generalized for mammals by Greenwood [6]. Hence, as shown in Fig. 2, the dependent variables were plotted against the same standardized logarithmic scale already used for comparison with other species elsewhere [9].

In the thalamus, only *CF*-related high DG uptake was observed; there was no response to 50 kHz. It occurred bilaterally in the ventral division of the principal nucleus of the medial geniculate body. Optical analysis revealed a 1.14-fold O.D. increase, when compared with less strongly labeled regions of the medial geniculate body (Fig. 3). Furthermore, contralaterally, the region of high DG uptake spread into the dorsal medial geniculate body. A part of the cerebral cortex exhibited a *CF*-evoked increase in DG uptake bilaterally (Fig. 3); densitometry revealed this to be slightly greater contralateral to the sound source.

None of the effects described above were seen in the unstimulated control animal. The stimulus-evoked response within the auditory system indicates a strong correlation of neuronal activity with tracer accumulation. Weak DG uptake of the cochlear nucleus and the superior olivary complex may have been due to drastic reduction of the metabolism by anesthesia [18] combined with the small size of the responding areas.

## DISCUSSION

The appearance of a lamina of high activity in the dorsolateral ICC after low frequency stimulation (Fig. 1A; white arrow) and a lamina of high activity in the ventromedial ICC after high frequency stimulation (Fig. 1B, white arrow) corresponds to findings from studies on gerbils [9] and cats [17] carried out with essentially the same methods applied here. In cats, single- and multi-unit recordings in the same animals used for DG studies demonstrated that a frequency-evoked band of high DG uptake in the ICC indeed contains neurons with *BFs* close to the frequency that evokes the band [17].

The location of frequency-evoked DG uptake (Fig. 1A and B; white arrows) agrees fairly well with the tonotopy derived from electrophysiological recordings [12] (Fig. 2). The faintness of the ICC/dl activation at 50 kHz was expected since the neurons there have higher thresholds [10]. Pollak and Schuller [12] claimed that the ICC/vm is exclusively reserved for *BFs* close to the individual bat's *CF*, i.e., 70–85 kHz. The *CF* isofrequency layer revealed by DG autoradiography is situated right in the center of ICC/vm (Fig. 1B; white arrow). Surprisingly, much of the ICC/dl, constituting almost 25% of ICC, responded to *CF*-stimulation, despite the fact that frequencies below 70 kHz are mapped onto this region (Fig. 1B; black arrow, and Fig. 2; open asterisk). The ICC/dl must have functional properties in addition to the mere processing of low frequency information ascending along the primary auditory pathway. In the cat, axon collaterals originating from various cell types in the region of the ICC/vm terminate in the region of the ICC/dl [11]. In the ICC of *Rhinolophus ferrumequinum*, intrinsic connections over such a distance have not been established yet. But axon collaterals of bipolar neurons form contacts with dendrites of other bipolar cells across isofrequency laminae [16]. Such intrinsic connections may account for the high frequency-evoked DG uptake in a low frequency region. Neuronal activity may occur there with such a delay that it escapes the time range of single- and multi-unit recording [12]. Extrinsic reciprocal connections with the reticular formation serve as another source of excitation in the IC. Their existence has been confirmed in *Rhinolophus ferrumequinum* [16]. In the ICC of the conscious rat, a dorsal and lateral, a central, and a ventral and medial band of high DG uptake were produced with electrical stimulation in a region of the reticular formation known to be linked with the IC also in the bat [4]. After conditioning of the rats, the reticular formation stimulation paired with 4–5 kHz sound sweeps increased the DG uptake in the three bands significantly [5]. Stimulation with sound sweeps alone produced only two bands of lesser DG uptake: one located dorsally and laterally, the other ventrally and medially in the ICC [4]. Hence, for each type of stimulus there is a distinct pattern of DG uptake in the ICC. If both stimuli are effective, the two patterns interact. In this study, the *CF* stimulus, which obviously is of more vital and alerting importance for the bat's sonar than the 50 kHz stimulus, could have triggered the reticular formation which in turn evoked DG uptake in the ICC/dl.

In anesthetized cats, inhibitory contours of reduced DG uptake were revealed in the ICC ipsilateral to the sound source [20]. One might have expected to find such contours in the echolocating bats, since at least half of the binaurally driven neurons in the superior olivary complex [7], the ICC [14], and the auditory cortex [8] respond with excitation when stimulated contralaterally, but with inhibition when stimulated ipsilaterally. However, in the cat experiments, neurons had to be activated by contralateral broad band noise and ipsilateral stimulation alone was insufficient to produce such contours, probably because the spontaneous activity was low [20]. The glucose consumption of an inactive neuron is apparently not reduced by its being further inhibited. The present failure to demonstrate an effect of ipsilateral inhibition on DG uptake may thus be attributed to low spontaneous activity in the ICC.

The strong contralateral bias in the responses, particularly in the ICC, confirms the strong lateral asymmetry of sound processing at the midbrain level. This is not primarily due to side differences in the strengths of the inputs, which are bilateral. Hence, the asymmetry must be produced by differences between the two sides in the complex spatiotemporal interactions between excitation and inhibition.

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