Brain Research Bulletin, Vol. 12, pp. 331–334, 1984. © Ankho International Inc. Printed in the U.S.A. 0361-9230/84 $3.00 + .00

BRIEF COMMUNICATION

Whole Head Sectioning in [^{3}H] Deoxyglucose Mapping of Auditory Responses in Gerbils

PETER MELZER

AK NRP, Zoologisches Institut der J.W. Goethe Universität, Siesmayerstr. 70
6000 Frankfurt am Main, FRG, and Institute of Anatomy, University of Lausanne
Rue du Bugnon 9, 1011 Lausanne, Switzerland

Received 26 August 1983

MELZER, P. *Whole head sectioning in [^{3}H] deoxyglucose mapping of auditory responses in gerbils.* BRAIN RES BULL 12(3) 331–334, 1984.—Stimulus-specific neuronal responses in the central auditory pathway of *Psammomys obesus* were studied with deoxyglucose autoradiography. Responses were revealed mainly in the inferior colliculus. With whole head sectioning and subsequent gentle freeze-drying, excellent structural preservation was achieved. With the use of double-tritiated deoxyglucose, Ultrofilm ^{3}H and vacuum-contact exposure, a mean spatial resolution of 43 μm Full Width Half Maximum was achieved.

Deoxyglucose autoradiography Whole body autoradiography Tritium Ultrofilm ^{3}H
Auditory pathway Psammomys obesus

IN recent years, deoxyglucose (DG) autoradiography has proven to be an efficient approach for assessing local cerebral metabolism and for mapping stimulus-specific responses throughout the entire brain [13]. In our laboratory, the technique has been employed to screen the central auditory pathway of the gerbil *Psammomys obesus* for neuron populations responding to a tone frequency (tonotopic organization [1]). In order to re-locate activated regions revealed with autoradiography in subsequent electrophysiological recording experiments, precise localization of regions of increased DG-uptake with minimum tissue deformation and shrinkage is required. I here describe a method which meets these requirements.

METHOD

Adult male sandrats, 8 to 12 weeks old, 127–277 g b.w., deprived of food for 24 hours prior to treatment, were anesthetized with 3 to 3.5% enflurane (Ethrane, Ohio Medical Products) applied by mask (oxygen flow ca. 2 l/min). Two animals served as non-stimulated controls, and three others were exposed to sound. Tone bursts were presented unilaterally in the free field (repetition rate 5/sec, burst duration 50 msec, rise and fall time 5 msec, frequency 2.5 kHz, 60 dB SPL). The right ear canal was occluded with a histoacrylate plug. Stimulation was begun simultaneously with intravenous injection of 2-[1,2-^{3}H]-deoxy-D-glucose (New England Nuclear) in saline (400 μCi/100 g b.w.) and continued for 45 minutes. Thereafter, animals were decapitated. Their heads were immediately embedded in carboxy-methyl cellulose and deep-frozen onto cryostat stages in liquid nitrogen.

Twenty micron thick frontal sections were cut on a 450 MP cryo-microtome (PMV) at −25°C, picked up on "Tesaband 206" adhesive tape (Beiersdorf), and gently freeze-dried in the microtome cabinet for 3 days. They were then exposed to Ultrofilm ^{3}H (LKB) in evacuated sealed light-proof plastic bags (vacuum-contact-technique [9]) at −25°C for 4 weeks. After adjustment to room temperature, film sheets were developed with a rapid X-ray film developer (ADEFO-Chemie) for 1 minute, rinsed in deionized water for 1 minute, fixed with X-ray film fixative (Tetenal) for 3 minutes, and hardened in running tap water for 10 minutes. All solutions were at room temperature. Since counterstaining of sections on tape could not be achieved with sufficient quality, autoradiographic structures were specified in comparison with Nissl-stained paraffin section series. Spatial resolution and optical densities were assessed with a microdensitometer (Zeiss) based on a Polyvar microscope (Reichert and Jung). It was set at three-fold magnification and apertures ranging from 20×50 μm for measuring spatial resolution to 50×150 μm for measuring optical densities. With this system, the autoradiogram area being measured could be identified visually at every moment of the scanning process. Gray levels of areas of interest were compared with those of contralateral and surrounding tissue by Student's

t-test (n=6); differences were taken to be significant with $p=0.01$, and optical density ratios were calculated for those cases. "Full Width Half Maximum" (FWHM)-values were determined directly from symmetric peaks in the densitometer readings. At sigmoidal optical density gradients, the distances between half-maxima and maxima were measured; doubling these distances gave FWHM-values, which could be pooled with those calculated for symmetric peaks.

RESULTS

Strong neuronal responses occurred in the inferior colliculus (IC, Fig. 1). In its central nucleus (ICC), activated fields of neurons shifted from dorsal to ventral with increasing tone frequencies. A detailed analysis of the effects of varying tone frequency is reported elsewhere (P. Melzer, submitted).

The spatial resolution achieved is demonstrated in Fig. 2. The anesthetic significantly activated neurons in the stratum granulosum areae dentatae (HI/sg) and the pyramidal stratum CA3 throughout the hippocampus [10]; the mean optical density level in these layers was twice that of surrounding tissue. Microdensitometer readings in the middle region (dorsoventrally) of HI/sg revealed a stratum image

FIG. 1. Partially enlarged autoradiogram of a frontal section from the posterior region of the IC. Stimulated side is on the left. White bar indicates 1000 μm. Note neuronal fields activated by the 2.5 kHz-stimulus in the ICC (white arrows). The contralateral was 1.3-fold higher than the ipsilateral activation.

FIG. 2. Bright-field photomicrographs of an autoradiogram (A) and a cresylecht violet-stained frontal paraffin section (B) from corresponding regions of the hippocampus. Increased accumulation of label is indicated in HI/sg (arrows).

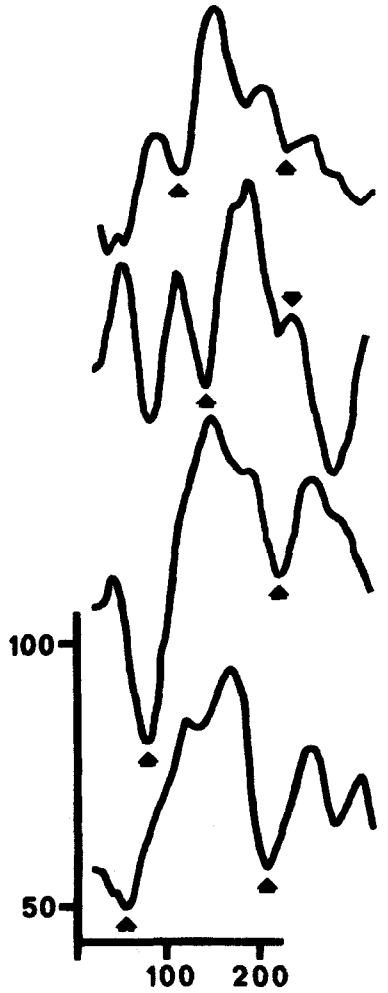

FIG. 3. Four typical densitometer readings from the autoradiographic image of the region displayed in Fig. 2. Absorbance (in percent; ordinate) was drawn as function of lateromedial tracing distance (μm; abscissa). The coordinate system applies to each of the curves, except that three of them have been displaced vertically. In each case, the peak is an absorption of 100%. The portions of the curves between the arrows correspond to HI/sg as judged visually during tracing. The slopes in that area range from 30 to 50 μm.

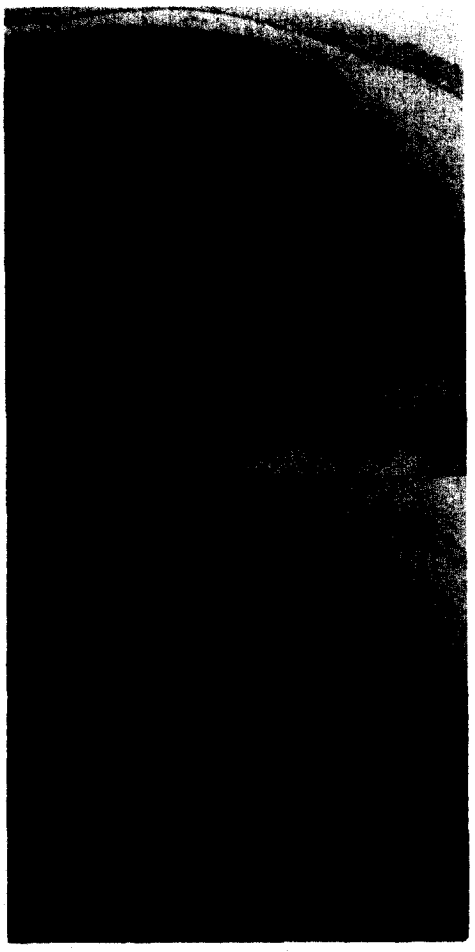

FIG. 4. Photomicrographs of frontal autoradiograms of a stimulated (A) and an unstimulated animal (B). Only the side contralateral to the sound source is displayed. Scale bar in A: 1000 μm. Bands of slightly increased DG-uptake in the cortex of the stimulated animal (arrows) correspond to layers II to IV and deep V.

FWHM of 70 μm and a mean FWHM for each border of 40 μm (Fig. 3). In this region, actual stratum width ranges between 40 and 60 μm (measured in frontal Nissl-stained paraffin sections and shrinkage corrected with a factor of 1.2), i.e. image blurring of only about 20–30 μm occurred here. Generally, a FWHM-mean of 43 μm (8 measurements per autoradiogram; S.D.=28.5%) was registered at sharply-edged structure discontinuities (for example gray/white matter boundaries in the cerebellum.

Activity discrimination is demonstrated in Fig. 4. Stimulus-specific activation was revealed in layers II to IV and deep V of both cortical hemispheres. Their activity increase was only 1.4-fold above adjacent areas, but it was significant ($p=0.01$; 10 measurements per autoradiogram).

DISCUSSION

The demonstration of bilateral activity bands in the ICC, responding to acoustic stimuli, has been observed in anesthetized cats [11], conscious rats [7], and another gerbilline species [12]. It is well consistent with the laminar tonotopic organization of that nucleus [1]. The pattern of cortical layer activation corresponds to findings in the conscious cat [8]. However, the boundaries of the auditory cortices could not be defined.

The tissue processing technique used was originally developed for macroautoradiography [14]. Whole head sectioning provided excellent tissue preservation and negligible shrinkage. The drying step seems to be the most crucial for spatial resolution in DG-autoradiography [5], and gentle freeze-drying considerably minimized tracer diffusion. Tritiated DG and Ultrofilm ^{3}H definitely added further to the improvement of spatial resolution [2,4]. By combining these approaches, we managed to achieve a spatial resolution well beyond the 100 μm of [^{14}C]DG-autoradiography [6] and close to the resolution limit demonstrated in bar phantom studies

with [^{3}H]DG and Ultrofilm ^{3}H [5]. A complication arises because of the low energy of ^{3}H beta particles, which leads to differences in self-absorption between white and gray matter [2]; but since the slopes of the tracer concentration/optical density functions for white and gray matter are very much alike [2], the higher absorption of radiation in white matter can be corrected. In any case, differential absorption would not affect the FWHM values. In contrast to techniques designed to reach even a cellular level of resolution [3], the method described here maintains the advantages of foil film contact autoradiography, i.e. the opportunity of exposing large numbers of low dose serial sections at reasonable expense of time and money.

ACKNOWLEDGEMENTS

This study was funded by the "Deutsche Forschungsgemeinschaft" (SFB 45, B 41). I am indepted to Dr. H. M. Kellner, Dr. G. Kloss, and Chr. Kötter, Hoechst AG, FRG, for helpful advice, and Dr. G. Fleissner, J. W. Goethe Universität, for giving me access to his densitometry equipment. I thank Dr. G. M. Innocenti and Dr. P. G. H. Clarke for reading the manuscript.

REFERENCES

1. Aitkin, L. M. Tonotopic organization at higher levels of the auditory pathway. In: *International Review of Physiology, vol 10, Neurophysiology II*, edited by R. Porter. Baltimore: University Park Press, 1976, pp. 249–279.
2. Alexander, G. M., R. J. Schwartzman, R. D. Bell, Jen Yu and A. Renthal. Quantitative measurement of local cerebral metabolic rate for glucose utilizing tritiated 2-deoxyglucose. *Brain Res* **223:** 59–67, 1981.
3. Durham, D., T. E. Woolsey and L. Kruger. Cellular localization of 2-[^{3}H]deoxy-D-glucose from paraffin-embedded brains. *J Neurosci* **1:** 519–526, 1981.
4. Faraco-Cantin, F., J. Courville and J. P. Lund. Methods for ^{3}H-2-D-deoxyglucose autoradiography on film and fine-grain emulsions. *Stain Technol* **55:** 247–252, 1980.
5. Gallistel, C. R. and S. Nichols. Resolution-limiting factors in 2-deoxyglucose autoradiography. I. Factors other than diffusion. *Brain Res* **267:** 323–333, 1983.
6. Goochee, C., W. Rasband and L. Sokoloff. Computerized densitometry and color coding of [^{14}C]deoxyglucose autoradiographs. *Ann Neurol* **7:** 359–370, 1980.
7. Huang, C., J. W. Dickson, J. Fex and L. Sokoloff. Patterns of local cerebral glucose utilization in the inferior colliculus of the rat. *Soc Neurosci Abstr* **11:** 57, 1981.
8. Hungerbühler, J. P., J. C. Saunders, J. Greenberg and M. Reivich. Functional neuroanatomy of the auditory cortex studied with [2-^{14}C]deoxyglucose. *Exp Neurol* **71:** 104–121, 1981.
9. Kloss, G., H.-M. Kellner and Chr. Kötter. Vakuum-Kontakt-Methode bei der Makroautoradiographie. *Z Naturforsch* **28c:** 468, 1973.
10. Myers, R. R. and H. M. Shapiro. Local cerebral metabolism during enflurane anesthesia: Identification of epileptogenic foci. *Electroencephalogr Clin Neurophysiol* **47:** 153–162, 1979.
11. Servière, J. and W. R. Webster. A combined electrophysiological and [^{14}C]2-deoxyglucose study of the frequency organization of the inferior colliculus of the cat. *Neurosci Lett* **27:** 113–118, 1981.
12. Sharp, F. R., A. F. Ryan, P. Goodwin and N. K. Woolf. Increasing intensities of wide band noise increase [^{14}C]2-deoxyglucose uptake in gerbil central auditory structures. *Brain Res* **230:** 87–96, 1981.
13. Sokoloff, L. Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. *J Cereb Blood Flow Metabol* **1:** 7–36, 1981.
14. Ullberg, S. Studies on the distribution and fate of S^{35}-labelled benzylpenicillin in the body. *Acta Radiol Suppl* **118:** 1–110, 1954.