CHANGES IN METABOLIC ACTIVITY IN THE INFERIOR COLLICULUS FOLLOWING REMOVAL OF AUDITORY INPUT

IKUO TANIGUCHI*

Department of Physiology, Dokkyo University School of Medicine, Mibu, Shimotsuga, Tochigi 321-02, Japan

ABSTRACT

Alteration of metabolic activity in the mouse inferior colliculus (IC) following cochlear destruction was studied by an autoradiographic 2- ^{14}C deoxyglucose (DG) technique. After unilateral cochlear destruction, DG uptake in the IC contralateral to the lesion decreased remarkably. DG uptake, however, began to increase as time passed and attained a normal level about one month later. A similar time course of recovery was also observed after bilateral cochlear destruction. On the other hand, the experiments in which the second cochlea was destroyed 8 months after the first demonstrated that the increase in DG uptake contralateral to the second lesion was confined to the external regions of the IC which receive somatosensory in addition to auditory input. These results suggest the augmentation of spontaneous activity in the IC neurons, and reinnervation by collateral sprouting from the somatosensory afferent in the IC.

KEY WORDS inferior colliculus / ^{14}C deoxyglucose technique / metabolic activity / cochlear damage / plastic changes

A recent autoradiographic 2- ^{14}C deoxyglucose (DG) technique developed by Sokoloff *et al.* (12) provides a pictorial representation of the rate of glucose consumption in macroscopic components of the brain. Since local neuronal activity is closely coupled to the rate of local glucose consumption, if functional alterations occur locally in the central nervous system in response to peripheral lesions, the DG technique can monitor the nature of the induced change in activity, and provide an estimate of its magnitude.

The DG technique has been used to demonstrate functional plasticity in the visual cortex of the immature monkey. The labelling of DG in ocular dominance columns completely disappeared as a consequence of early monocular visual deprivation. This phenomenon was interpreted in terms of preemption of the dom-

inance columns for the deprived eye by those for the intact eye (4). It is generally recognized that a group of neurons in the central nervous system which has been deprived of its major input begins to respond to a novel input after a few days (6, 16).

Our study is concerned mainly with the alteration of metabolic activity in the inferior colliculus (IC) following cochlear destruction. In a previous paper (13) we reported that after unilateral cochlear destruction a remarkable decrease in the rate of glucose consumption occurred in the IC contralateral to the lesion. This result is consistent with previous neuroanatomical (7) and DG autoradiographic (11) studies indicating that the IC is predominantly innervated by the crossed afferent fibers. As time passed after the cochlear destruction, however, the rate of glucose consumption increased in the contralateral IC. The present study supports and extends the previous observation. When the second cochlea was destroyed 8

*Present address: Department of Biology, Washington University, St. Louis, MO 63130, U.S.A.

months after the first to evaluate the contribution of the uncrossed afferent fibers to the recovery of metabolic activity, an unexpected result was obtained. Furthermore, even if the normal peripheral auditory inputs were completely lost by simultaneous bilateral cochlear destruction, recovery occurred in the IC on both sides. Possible explanations for these results are discussed.

MATERIALS AND METHODS

A total of 30 immature ICR mice, weighing about 15 g, was used. They were divided into four groups with different cochlear lesion histories, i.e. (1) with both cochleas intact, (2) with one cochlea destroyed 1 day to 8 months (1, 4, 10, 41 days and 8 months) before the experimental manipulation, (3) with one cochlea destroyed 8 months before, and the remaining cochlea destroyed 1 day before the experimental manipulation, and (4) with both cochleas destroyed 1 to 40 days (1, 4, 10, 20 and 40 days) before the experimental manipulation. At least two animals were used for each subsequent experimental manipulation. Unilateral or bilateral cochlear destruction was carried out at 3 weeks of age under intraperitoneal sodium pentobarbital anesthesia (3 mg/100 g body weight). The relative rate of glucose utilization in the IC was observed by the autoradiographic DG technique after the different cochlear lesions described above.

2-Deoxy-D- $[^{14}\text{C}]$ -glucose (specific activity, 53 mCi/mmol) was obtained from New England Nuclear Corp., evaporated, and reconstituted in sterile saline. DG was administered intravenously through the tail vein as a pulse (2 μCi /10 g body weight in 100 $\mu\text{Ci}/\text{ml}$ saline) to the alert and unanesthetized animal. After injection, each animal was held unrestrained in a cage (20 cm $\times$ 15 cm $\times$ 15 cm high) in a sound-proof room, with or without sound stimulation. Forty-five minutes after the DG injection, the animal was sacrificed by the intraperitoneal administration of a large dose of sodium pentobarbital and decapitated. The brain was quickly removed and placed in chilled embedding medium (Lab-Tek Products, Tissue-Tek II) in a small metal container (2 cm $\times$ 3 cm $\times$ 2 cm deep) and frozen in acetone cooled with dry ice.

The frozen brain was mounted with embedding medium on a cryostat (Nakagawa, NA 300) maintained at -15°C , and frozen sections 30 μm thick were cut. Sections were picked up on

glass slides and immediately dried on a hot plate at $50\text{--}60^\circ\text{C}$. The sections were then exposed to single emulsion X-ray film (Sakura MARG, ^3H type) in X-ray film cassettes for 2 days, and the film was developed according to the instructions supplied with it.

The optical density of the autoradiographs was measured by a densitometer (JEOL, JCR-80) with an aperture of 0.2 mm, which was controlled by a computer (Digital Equipment Corporation, PDP 11-40). Twenty-five readings of light transmittance were averaged for the IC and the commissure of IC. The optical density for the IC was expressed relative to that for the opposite IC or the commissure of IC.

Fig. 1 Autoradiographs of coronal brain sections at the level of the IC of a mouse with normal auditory function. Note that auditory nuclei are selectively labelled with DG. SO: superior olivary complex; NLL: nucleus of lateral lemniscus; IC: inferior colliculus

For sound stimulation, a white noise burst was generated by a random noise generator (General Radio, 1382) through an electronic switch (Nagashima Medical Instruments, Na 1030). A stimulus train composed of 10 noise bursts was repeated at intervals of 10 sec. The duration, rise-fall time and interval of each burst were 100 msec, 10 msec and 400 msec, respectively. The sound stimulus was delivered by a loud speaker (Mitsubishi Electric, R 305) which was located 1 m in front of the animal cage in the sound-proof room. The intensity of the stimulus was fixed at 60 dB SPL, which was calibrated with a 1" condenser microphone (Blüel and Kjaer, 4145) and frequency analyzer (Blüel and Kjaer, 2120).

RESULTS

Normal Binaural Hearing

Autoradiographs shown in Fig. 1 are representative coronal sections at the level of the IC of a mouse with both intact cochleas exposed to a white noise burst. The high optical density in activated regions is due to the increased uptake of DG. While there was non-specific labelling in structures such as the vestibular system, clear selective labelling was apparent in the auditory system: the cochlear nucleus (CN), the superior olivary complex (SO), the nuclei of the lateral lemniscus (NLL), and the IC. In the IC, the presence of selective labelling was usually even more striking than in other regions.

We have sometimes failed to obtain selective

Fig. 2 A: Autoradiographs at the level of the IC of the mice after unilateral destruction of the left cochlea. Autoradiographs were obtained at different postoperative days, from 1 day to 8 months (a: 1, b: 4, c: 10, d: 20, e: 41 days, f: 8 months). Note that reduced DG uptake in the right IC contralateral to lesion as indicated by an arrow gradually increases over time. B: The time course of increase in the optical density for the right IC relative to that for the left (R/L). Letters a to f correspond to those in A.

labelling at the level of either the medial geniculate body or the auditory cortex. A similar failure in cats has been reported by Webster *et al.* (18). Differential labelling in the subdivisions of the auditory nuclei was also not obvious because it was quite difficult to obtain good resolution in the small brain of the mouse used.

Unilateral Cochlear Destruction

After unilateral cochlear destruction, autoradiographs were made at the level of the IC at different postoperative intervals (Fig. 2A). One day after the unilateral destruction of the left cochlea, the IC contralateral to the lesion exhibited markedly less labelling than did the ipsilateral side (Fig. 2A, a).

After a few days, however, the optical density of the contralateral IC gradually increased resulting in less asymmetry of DG uptake. On the 10th postoperative day, bilateral asymmetry was apparently reduced (Fig. 2A, c). Furthermore, on the 20th and the 41st postoperative days optical densities increased symmetrically in the IC and showed essentially an equal uptake of DG on both sides (Fig. 2A, d and e). This time course is very similar to that observed in the previous study (13). On the autoradiograph at 8 months after unilateral cochlear destruction, a reversed asymmetry was demonstrated, that is, DG uptake was larger in the IC contralaterally than ipsilaterally (Fig. 2A, f). A similar ten-

dency was observed in another animal on the same postoperative day.

Fig. 2B shows quantitatively the time course of increase in DG uptake in the IC contralateral to the lesion. The optical density for the right IC is expressed relative to that for the left (R/L). A marked asymmetry of optical densities in the IC on the 1st postoperative day is expressed with greater clearness in terms of the ratio. The ratio gradually increases with postoperative days and after 8 months it attains a value above the normal, resulting in a reversed asymmetry.

Additional Destruction of Remaining Cochlea

From the results described above, we assumed that the ipsilateral input that normally exists might begin to contribute significantly to activation of the IC after destruction of the contralateral afferent input which is normally predominant. To investigate this, an additional surgical operation was made on the remaining right cochlea 8 months after the unilateral destruction of the left cochlea. If the activity of the IC is enhanced by the ipsilateral input, destruction of the remaining cochlea may result in a remarkable decrease in DG uptake.

Unexpectedly, the results did not support this assumption. The IC ipsilateral as well as contralateral to the new lesion did not exhibit a marked decrease in DG uptake, as shown in Fig. 3. The whole area of the IC was diffusely

Fig 3 A: Autoradiograph at the level of the IC of the mouse after destruction of each cochlea at an interval of 8 months. The right cochlea destroyed for 1 day after unilateral destruction of the left cochlea 8 months before. Note that DG uptake in the central region of the IC contralateral to new lesion is less densely labelled than the surrounding zones. B: Regional optical densities for the left IC are shown relative to that for the central region of the right IC.

labelled. The central region of the left IC contralateral to the new lesion, however, is less densely labelled than the surrounding zones. In Fig. 3B the optical densities for the central region and the surrounding zones of the IC are expressed relative to that for the central region of the right IC. The optical density for the central region of the left IC fell to about one half of the optical densities for the surrounding zones. This indicates that the central nucleus of the IC is predominantly innervated by the crossed afferent fibers.

Bilateral Cochlear Destruction

The observation that the IC contralateral to the

new additional lesion in the remaining cochlea did not show any remarkable decrease in DG uptake (Fig. 3) led us to investigate the possibility that the IC may recover normal levels of metabolic activity even if auditory inputs are completely removed. This was confirmed by autoradiography following simultaneous destruction of both cochleas (Fig. 4).

The autoradiograph on the 1st postoperative day showed a remarkable decrease in DG uptake on both sides of the IC (Fig. 4A, a). On subsequent days, however, the uptake of DG increased symmetrically. On the 20th postoperative day the uptake in the IC reached essentially the normal level bilaterally (Fig. 4A, d). The time courses of the increase in DG

Fig. 4 A: Autoradiographs at the level of the IC of the mice after bilateral cochlear destruction. Autoradiographs were obtained at different postoperative days, from 1 day to 40 days (a: 1, b: 4, c: 10, d: 20, e: 40 days). Note that bilaterally reduced DG uptake in the IC gradually increases over time. B: The time course of increase in the optical density for the right and the left IC relative to that for the commissure of IC (R/C, L/C). Letters a to e correspond to those in A. An asterisk indicates the ratio measured on the autoradiograph of the normal animal shown in Fig. 1.

uptake in the IC on both sides are quantitatively shown in Fig. 4B. The optical densities for the IC on both sides are expressed relative to that for the commissure of IC (L/C, R/C). The curves of ratios L/C and R/C are closely superimposed. Immediately after cochlear destruction the ratio was about 1.0 and a gradual increase in the ratio then followed. After 40 days the ratio attained the normal level or beyond. This time course is quite similar to that observed after unilateral cochlear destruction. Apparently the neurons of the IC become active even without the auditory afferent input.

DISCUSSION

The present study shows that the IC can restore its metabolic activity even if auditory peripheral inputs are completely removed by bilateral cochlear destruction. In addition the time course of recovery after bilateral cochlear destruction is quite similar to that after unilateral cochlear destruction. These results suggest that spontaneous activity in the auditory neurons of the IC increases when normal inputs from the cochlea are removed.

Under normal conditions, many neurons of the IC fail to discharge spontaneously. Such an inhibitory control may be exerted not only by the afferent fibers, but also by the corticofugal efferent fibers to the IC (17). Birt *et al.* (2) studied the effect of bilateral ablation of the auditory cortex on unit activity in the rat IC. In the lesioned animals, spontaneous activity in the IC neurons increased. They favored the idea that a non-specific activation from a non-cortical structure is selectively antagonized by cortical efferents. In animals with cochlear lesions, however, cortical suppression may not be exerted since the cortex is supposed to be affected transsynaptically following cochlear destruction. In the visual system, Globus and Scheibel (5) showed a reduction in the number of dendritic spines of cortical neurons in rabbits after unilateral enucleation.

Abnormally enhanced excitability in the IC has also been demonstrated after exposing mice to a brief but intense sound during a certain developmental period (15). This initial sound exposure, termed acoustic priming, subsequently renders animals extremely susceptible to audiogenic seizures. The IC neurons in primed animals were characterized by prolonged discharges in response to clicks, and after-discharges in response to tones. A recent study by Tepper and Schlesinger (14) showed cochlear receptor

damage induced in primed mice. This supports the hypothesis that receptor damage leads to the development of supersensitivity in more central auditory nuclei. An electrophysiological study by Silverman and Clopton (10) demonstrated that inhibition in the IC, typically evoked by stimulation of the ipsilateral ear, was absent in monaurally deprived rats. It is conceivable that some type of disinhibition results in an increased metabolic activity in the IC following peripheral damage.

Many authors since Cajal (3) have divided the IC into three subdivisions: central, pericentral and external nuclei. The first two of these are undoubtedly auditory. The external nucleus has been recognized as receiving somatosensory projections from the dorsal column nuclei (9). Recently, auditory and somatosensory properties of the external nucleus of the cat IC were electrophysiologically studied by Aitkin *et al.* (1). They showed that many neurons in this region receive a bimodal input, while a small proportion of the total population was only excited by auditory stimulation. Of the neurons responding to both acoustic and spinal inputs, a majority were excited by tonal stimuli, and the tone response was inhibited by electrical stimulation of the dorsal columns.

The results of our experiments in which the second cochlea was destroyed 8 months after the first indicated that the increase in DG uptake contralateral to the second lesion was confined to the external regions of the IC rather than the central. On the other hand, immediately after either unilateral or bilateral cochlear destruction, a remarkable reduction in DG uptake was observed in the whole area of the IC. These results indicate that the bimodal neurons in the IC normally need an auditory input to function. If however the auditory input to the bimodal neurons is lost as a result of cochlear damage, the remaining somatosensory input may begin to activate the neurons.

If the synaptic sites in the IC neurons, where the axons from the lower auditory nuclei terminate, are transsynaptically degenerated by cochlear destruction, reinnervation by collateral sprouting of the somatosensory neurons may occur, occupying the evacuated sites and causing restoration of DG uptake in the IC. Raisman and Field (8) have demonstrated the occurrence of reinnervation of denervated sites in the septal nuclei of rats. After section of the ipsilateral fimbria the number of non-degenerating spine synapses decreased to about half. The number began to rise again however and by one month

later there were the same numbers of synapses on spines as in the normal animal. Their explanation for this was the reinnervation of denervated sites by adjacent non-degenerating axon terminals. This time course is quite similar to that of the increase in DG uptake observed in our study.

The possible mechanisms described above, i.e., the augmentation of spontaneous activity, supersensitivity and reinnervation, are not mutually exclusive. Although further studies are needed to clarify this problem, our results may present a new aspect of plastic reorganization in the central nervous system of mammals.

The author wishes to thank Professors Y. Katsuki and N. Saito for their support, and Mr K. Kimura for his help in densitometry. The critical comments of Professor R. Norgren improved the manuscript, and are greatly appreciated.

Received for publication 30 August 1980

REFERENCES

1. AITKIN L. M., DICKHAUS H., SCHULT W. and ZIMMERMANN M. (1978) External nucleus of inferior colliculus: auditory and spinal somatosensory afferents and their interactions. *J. Neurophysiol.* **41**, 837-847
2. BIRT D., NIENHUIS R. and OLDS J. (1978) Effects of bilateral auditory cortex ablation on behavior and unit activity in rat inferior colliculus during differential conditioning. *J. Neurophysiol.* **41**, 705-715
3. CAJAL S. RAMÓN Y. (1911) *Histologie du Système Nerveux de l'Homme et des Vertébrés*. Maloine, Paris
4. DES ROSIERS M. H., SAKURADA O., JEHL J., SHINOHARA M., KENNEDY C. and SOKOLOFF L. (1978) Functional plasticity in the immature striate cortex of the monkey shown by the [^{14}C]deoxyglucose method. *Science* **200**, 447-449
5. GLOBUS A. and SCHEIBEL A. B. (1966) Loss of dendrite spines as an index of pre-synaptic terminal patterns. *Nature* **212**, 463-465
6. MERRILL E. G. and WALL P. D. (1978) Plasticity of connections in the adult nervous system. In *Neuronal Plasticity* (ed. COTMAN C. W.) Raven Press, New York, pp. 97-111
7. OSEN K. K. (1969) The intrinsic organization of the cochlear nuclei in the cat. *Acta Otolaryngol.* **67**, 352-359
8. RAISMAN G. and FIELD P. M. (1973) A quantitative investigation of the development of collateral reinnervation after partial deafferentation of the septal nuclei. *Brain Res.* **50**, 241-264
9. ROBARDS M. J. (1979) Somatic neurons in the brainstem and neocortex projecting to the external nucleus of the inferior colliculus: an anatomical study in the opossum. *J. Comp. Neurol.* **184**, 547-566
10. SILVERMAN M. S. and CLOPTON B. M. (1977) Plasticity of binaural interaction. I. Effect of early auditory deprivation. *J. Neurophysiol.* **40**, 1266-1274
11. SOKOLOFF L. (1976) Functional mapping by the DG method: auditory stimulation. *Neurosci. Res. Prog. Bull.* **14**, 489
12. SOKOLOFF L., REIVICH M., KENNEDY C., DES ROSIERS M. H., PATLAK C. S., PETTIGREW K. D., SAKURADA O. and SHINOHARA M. (1977) The [^{14}C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J. Neurochem.* **28**, 897-916
13. TANIGUCHI I. and SAITO N. (1978) Plastic reorganization in the inferior colliculus of the immature mouse studied by [^{14}C]deoxyglucose method. *Proc. Japan Acad.* **54**, 496-499
14. TEPPER J. M. and SCHLESINGER K. (1980) Acoustic priming and kanamycin-induced cochlear damage. *Brain Res.* **187**, 81-95
15. URBAN G. P. and WILLOTT J. F. (1979) Response properties of neurons in inferior colliculi of mice made susceptible to audiogenic seizures by acoustic priming. *Exp. Neurol.* **63**, 229-243
16. WALL P. D. and EGGER M. D. (1971) Formation of new connexions in adult rat brains after partial deafferentation. *Nature* **232**, 542-545
17. WATANABE T., YANAGISAWA K., KANZAKI J. and KATSUKI Y. (1966) Cortical efferent flow influencing unit responses of medial geniculate body to sound stimulation. *Exp. Brain Res.* **2**, 302-317
18. WEBSTER W. R., SERVIERE J., BANTINI C. and LAPLANTE S. (1978) Autoradiographic demonstration with 2- ^{14}C deoxyglucose of frequency selectivity in the auditory system of cats under conditions of functional activity. *Neurosci. Lett.* **10**, 43-48