CYTOCHEMICAL INVESTIGATION OF BINAURALLY CONVERGING CONNECTIONS IN NUCLEI OF THE CAT SUPERIOR OLIVE

G. N. Shmigidina and I. N. Kudryavtseva

UDC 612.828.015.2:547.963.32

Binaurally converging connections at the level of the superior olivary nuclei were investigated in anesthetized cats by the method of quantitative cytospectrophotometry. As a result of monaural stimulation for 2 h with burst of rhythmic noise a marked increase was observed in the content of cytoplasmic RNA in neurons of the ipsilateral and contralateral medial and lateral nuclei. The volume of functioning neurons of these nuclei either showed no significant change or was increased. Some idea of the character of the functional organization of binaurally convergent connections can be deduced from these results.

KEY WORDS: superior olivary nuclei; cytoplasmic RNA; binaurally convergent connections.

According to morphological and electrophysiological observations the nuclei of the superior olivary complex are the first level for convergence of afferentation from the right and left ears [1, 6]. It is therefore by investigation of cytochemical changes in the superior olivary complex that the functional organization of binaurally convergent connections at this level of the auditory system can be judged.

The object of this investigation was to make a quantitative assessment of the RNA content in neurons of the superior olivary complex in the cat.

EXPERIMENTAL METHOD

Experiments were carried out on ten anesthetized (chloralose and urethane, 30 and 500 mg/kg, respectively) cats (5 experimental and 5 control animals). The cats were kept in a soundproof chamber. Acoustic volleys, 15 msec in duration, with a repetition frequency of 25 Hz and acting for 2 h, were used as stimuli. The acoustic pressure at the output of the telephone was 80 dB above the $2 \cdot 10^{-5}$ N/m^2 level. The sound generator was a TD-6 telephone. Material was fixed in Carnoy's fluid and embedded in paraffin wax. Sections 7 μ in thickness were stained with gallocyanin–chrome alum [5]. The RNA concentration was found by the equation $C = E/\chi_\lambda d$ [3]. The optical density of the neuron cytoplasm was measured by means of the MUF-5 two-wavelength recording probe cytospectrophotometer. To calculate the volume of the neuron cytoplasm the equation for an ellipsoid of rotation was used: $V = \pi/6\ Dd^2$, where D is the greater diameter and d the lesser diameter. The RNA content was determined by the equation $Q = C \cdot V = E_V/\chi_\lambda \cdot d$, the product $\chi_\lambda \cdot d$ being taken as a constant. The error of the RNA content was calculated by the equation: $\sigma_Q = \pm Q\sqrt{(\sigma\, c/C)^2 + (\sigma_V/V)^2}$ [3]. Concentration was determined from measurements on 160-180 cells.

EXPERIMENTAL RESULTS AND DISCUSSION

As the data given in Table 1 show, the RNA content in neurons of the medial and lateral nuclei of the superior olivary complex was higher than in the control after acoustic stimulation both on the ipsilateral (right) and on the contralateral (left) side relative to the stimulated ear. The distribution of the RNA content was normal in character. There was a marked increase in the RNA content in neurons of the caudal

Laboratory of Physiology of Hearing and Laboratory of Functional Neurchemistry, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 2, pp. 242-244, February, 1976. Original article submitted May 25, 1975.

TABLE 1. Concentration and Total Content of Cytoplasmic RNA and Cytoplasmic Volume of Neurons of Lateral and Medial Nuclei During Monaural Acoustic Stimulation (M ± m)

Nucleus	Side	Part	Control			Acoustic stimulation		
			RNA concentration (in log of optical density)	cytoplasmic volume (in nm³)	RNA content calculated per cell (rel.un.)	RNA content calculated per cell rel. un.)	cytoplasmic volume (in nm³)	RNA content calculated per cell (in relative units)
Medial	Ipsilateral	Rostral	0,36±0,008	2513±261	905±99	0,48±0,005*	2708±113	1300±52*
		Central	0,39±0,009	2118±294	826±33	0,50±0,007*	2820±122†	1410±59*
		Caudal	0,38±0,011	2293±170	871±68	0,49±0,007*	2885±220†	1423±99*
	Contralateral	Rostral	0,35±0,008	2481±192	868±61	0,48±0,007*	2433±240	1168±105*
		Caudal	0,35±0,008	2510±203	879±70	0,46±0,007*	2809±262	1292±116*
		Cuadal	0,33±0,008	2475±241	817±90	0,47±0,007*	2935±108	1379±14*
Lateral	Ipsilateral	Rostral	0,633±0,008	2473±92	816±33	0,43±0,008*	3447±361	1482±148*
		Central	0,32±0,007	2229±236	713±71	0,48±0,008*	2693±235	1295±104*
		Caudal	0,31±0,013	2548±138	790±50	0,48±0,008*	3040±263	1459±117*
	Contralateral	Rostral	0,34±0,007	1885±175	641±58	0,43±0,009*	3118±233*	1340±94*
		Central	0,31±0,007	2113±254	655±79	0,38±0,008*	2684±256	1046±94*
		Cuadal	0,31±0,007	1887±228	585±70	0,38±0,011*	2736±256	1040±95*

* Compared with control P < 0.0001.
† Compared with control P < 0.003.

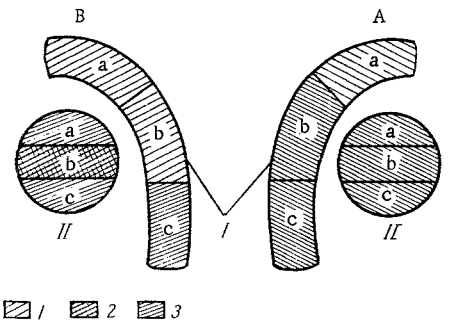

Fig. 1. Scheme of medial (I) and lateral nuclei (II): distribution of afferent fibers in different parts of nuclei based on RNA content as a percentage of control level: 1) deviation by 40-50%; 2) by 50-60%; 3) by 60% or over. A) Ipsilateral side; B) contralateral side relative to stimulated ear; a) rostal, b) central, c) caudal part of nucleus.

and central parts of the left medial nucleus. In the right and left lateral nuclei, substantial increases also were found in the RNA content in the rostral, central, and caudal parts, except in the central part of the left lateral nucleus, in which deviations from the control level were smaller than in the remaining parts of that nucleus.

The volume of the cytoplasm of the neurons in the medial and lateral nuclei either did not differ significantly from the volume in the control and experimental series or it was decreased as a result of stimulation (Table 1).

The greatest deviation from the corresponding control level, which was observed in the caudal and central parts of the ipsilateral medial nucleus and in the caudal part of the contralateral medial nucleus, is evidence of the distribution of afferentation from the right and left ears predominantly on neurons of these parts of the medial nuclei (Fig. 1). The increase in the RNA content in neurons of the ipsilateral and contralateral lateral nucleus compared with the corresponding control level suggests a relatively uniform distribution of afferent fibers on neurons of the lateral nuclei (Fig. 1).

For the overwhelming majority of cells of the medial nucleus, contralateral stimulation is known to be excitatory whereas ipsilateral stimulation has an inhibitory action if spike activity is tested [1, 6]. The fact that an increase in RNA content was observed in both types of monaural stimulation suggests that the development of hyperpolarization on the neuron membrane even evoke accumulation of RNA.

So far as the lateral nucleus of the superior olivary complex is concerned, a more marked increase in RNA was observed in the neurons composing it in response to ipsilateral than contralateral stimulation. It will be noted that in the lateral nucleus it is ipsilateral stimulation that is excitatory whereas contralateral stimulation has an inhibitory effect [11].

This investigation thus indicates that the RNA content is also increased in neurons of the auditory system during function; this confirms data (qualitative) in the literature obtained by the investigation of receptor cells of the organ of Corti [4] and the quantitative data obtained by investigation of neurons of the spiral ganglion [7-10] and also of the cochlear nuclei [12]. In addition, this investigation has shown that

cytospectrophotometry is a promising method for the study of the functional organization of connections, at least for the investigation of binaural convergence of afferentation.

LITERATURE CITED

1. Ya. A. Al'tman, Localization of Sound. Neurophysiological Mechanisms [in Russian], Leningrad (1972).
2. F. M. Bakharev, I. I. Davydova, I. A. Zarubina, et al., Tsitologiya, No. 6, 114 (1964).
3. V. Ya. Brodskii, Cell Nutrition [in Russian], Moscow (1966).
4. Ya. A. Vinnikov and L. K. Titova, The Organ of Corti. Histophysiology and Histochemistry [in Russian], Moscow–Leningrad (1961).
5. L. Einarson, Acta Path. Microbiol. Scand., 28, 82 (1951).
6. C. D. Erulcar, Physiol. Rev., 5, 237 (1972).
7. O. Hallen, J. E. Edström, and A. Hamberg, Acta Oto-Laryng. (Stockholm), 60, 121 (1965).
8. G. Hammer, Acta Oto-Laryng. (Stockholm), Suppl. 127, 1 (1956).
9. G. Hammer, Acta Oto-Laryng. (Stockholm), Suppl. 140, 137 (1958).
10. E. Thomsen and H. Pakkenberg, Acta Oto-Laryng. (Stockholm), 55, 260 (1962).
11. C. Tsuchitani and J. C. Boudreau, J. Neurophysiol., 29, 684 (1966).
12. E. Wüstenfeld, H. N. O. (Berlin), 20, 262 (1972).