

## DISTRIBUTION OF GAMMA-AMINOBUTYRIC ACID, GLYCINE, GLUTAMATE AND ASPARTATE IN THE COCHLEAR NUCLEUS OF THE RAT<sup>1</sup>

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Received for publication September 20, 1977, and in revised form October 11, 1977

**The distributions of  $\gamma$ -aminobutyric acid (GABA), glycine, glutamate and aspartate were measured in cochlear nuclei of two rats by quantitative histochemical mapping procedures. The levels and distributions in the two rats were comparable, and resembled those previously reported for cat cochlear nucleus. The results are consistent with a concept that these putative transmitter amino acids have similar levels and distributions in the cochlear nucleus among mammals.**

While extending a study of the distribution of candidate transmitter amino acids in the cochlear nucleus of the cat (8), some data were also obtained for the rat cochlear nucleus. These data are reported here for comparison with those of the cat. Anatomical (5, 11-13) and physiological (17-19) studies on rat cochlear nucleus indicate similarities with the cat, but chemical data have been scarce (6).

### METHODS

The procedures for obtaining tissue samples and carrying out chemical assays were the same as those used for studying the cat cochlear nucleus (8) and have been previously described (3, 9, 15).

The data are from the right cochlear nuclei of two male albino Sprague-Dawley rats. Rat A (121172)<sup>2</sup> was sacrificed, without anesthesia, by decapitation such that the head dropped directly into Freon-12 chilled to its freezing point with liquid nitrogen. Tissue blocks containing the cochlear nuclei were subsequently isolated from the head maintained at  $-20^{\circ}\text{C}$ . Rat B (022972A) was sacrificed by bleeding while under sodium pentobarbital anesthesia; the brain was dissected out and frozen in Freon-12 at its freezing point. Approximately 40 min elapsed between death and freezing.

Tissue blocks were sectioned at  $-20^{\circ}\text{C}$ , and the sections were freeze-dried. Samples dissected from the freeze-dried sections were weighed, loaded into oil-

covered 1- $\mu\text{l}$  droplets of 0.05N NaOH, then heated at  $80^{\circ}\text{C}$  for 20 min to destroy enzyme activities. In most cases, four separate aliquots were subsequently taken from each droplet for fluorometric assays of GABA, glycine, glutamate and aspartate levels, but in some cases only GABA and glycine were measured.

### RESULTS

The distributions of the amino acids in the cochlear nuclei of rats A and B are presented on a dry weight basis (Figs. 1-4). Dry weight is the reference quantity most accurately measured with the procedures employed, and was used previously for presenting the distributions in the cat cochlear nucleus (8). The distributions for rat A can also be appreciated on a volume basis by using the data of Figure 5. Volume is probably a more relevant reference quantity for the amino acids since it relates closely to the lipid-free fraction of the tissue (9, 16), where the bulk of the amino acids are likely to be. The volume basis is used for comparing the averaged regional data for the two rats (Table I) as well as the comparison of rat and cat cochlear nuclei (Table II).

The data for rats A and B were generally similar with only a few notable exceptions. GABA levels were higher, usually about 40% higher, in rat B than in rat A, in regions other than fiber tracts. Such a difference between the two rats is not surprising in view of previously reported post-mortem increases of GABA levels in brain tissue (2, 14, 20, 22). Glycine levels were higher in the posteroventral cochlear nuclear subdivision of rat B than in that of rat A; glutamate levels were higher in the molecular and fusiform-cell layers of rat A than in those of rat B. It is of course impossible from the present

<sup>1</sup> These studies were supported by the American Cancer Society through Research Grant BC4Q and the National Institutes of Health through Research Grants NS08000 and NS08862.

<sup>2</sup> The numbers in parentheses identify the animals by date of sacrifice as previously described (9). Although the shorter identification as rat A or B will be used here, the date identifications enable comparison of the amino acid data with other chemical data on the same animals to be presented elsewhere.

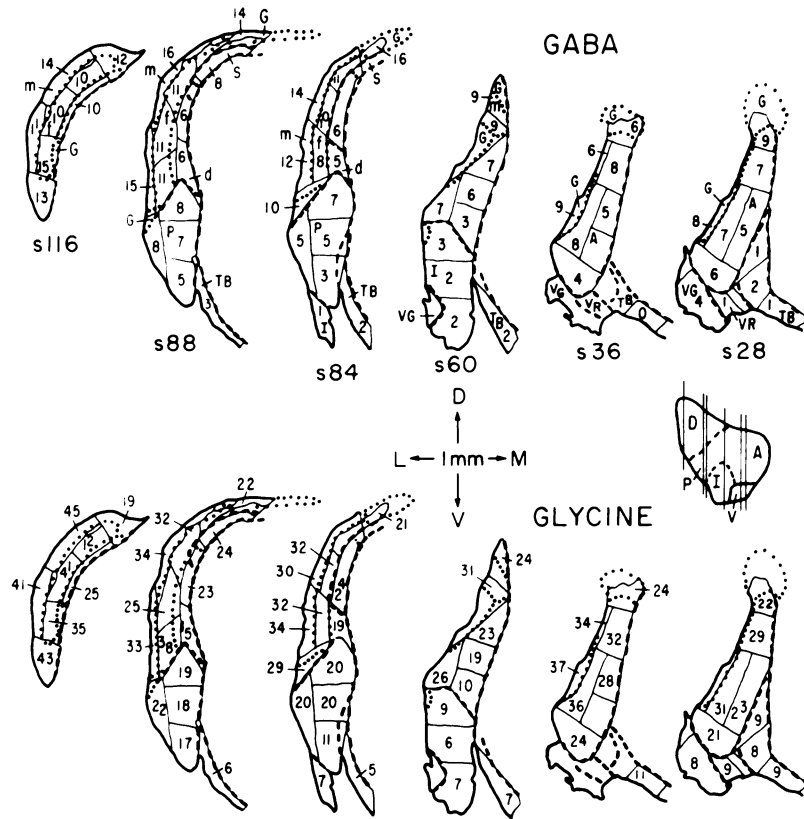


FIG. 1. Levels of  $\gamma$ -amino butyric acid (GABA) and glycine, in mmoles/kg dry weight, in transverse  $20\ \mu\text{m}$ -thick sections through the cochlear nucleus of rat A. The approximate locations of the sections are indicated on the side view drawing of a rat cochlear nucleus, as described previously (9). The scale and directional arrows pertain to the sections. In the section drawings, thin lines are the sample boundaries, while solid, dashed and dotted thick lines are regional boundaries. Among the regional boundaries, dashed lines are more approximate than solid lines, and dotted lines were drawn by reference to nearby ( $40\ \mu\text{m}$  away) thionin-stained sections. Abbreviations are: A, anteroventral cochlear nucleus; D, dorsal cochlear nucleus; G, granular region; I, interstitial nucleus; m, f, d, molecular, fusiform-cell and deep layers of dorsal cochlear nucleus; P, posteroventral cochlear nucleus; S, acoustic striae; TB, trapezoid body; VG, vestibular nerve ganglion, VR or V, vestibular nerve root.

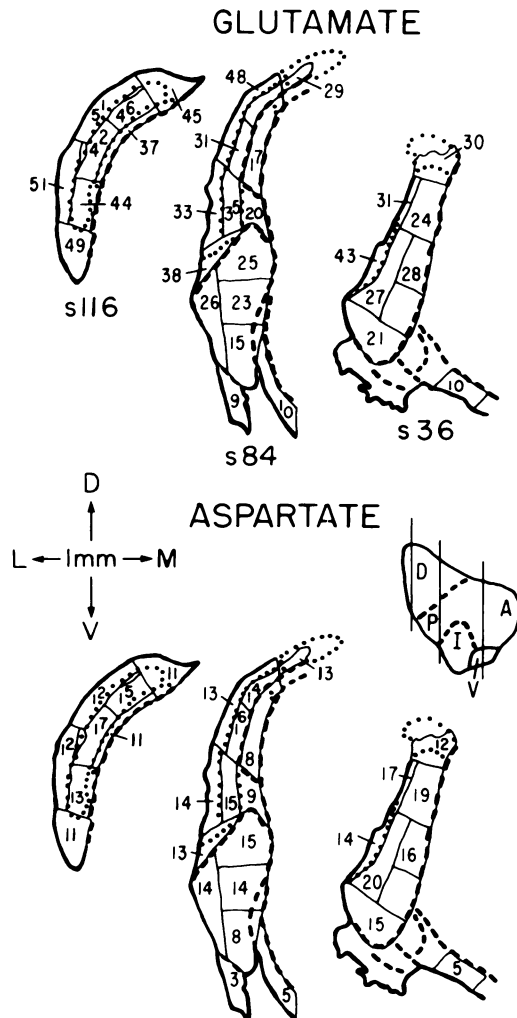


FIG. 2. Levels of glutamate and aspartate, in mmoles/kg dry weight, in transverse sections through the cochlear nucleus of rat A. These are the same sections as three of those in Figure 1 as indicated by the section numbers. Details as in Figure 1.

study to attach any significance to these differences.

For all four amino acids, the lowest levels within the cochlear nucleus were in the interstitial nucleus. The highest levels of GABA were in the molecular and fusiform-cell layers of the dorsal cochlear nucleus, whereas, for the other amino acids, the high levels were more generally distributed. The ratios on a dry weight basis between highest and lowest levels found in the parts of the cochlear nucleus other than the interstitial nucleus were 4-5 for GABA, 2-4 for glycine, and 2-3 for glutamate and aspartate. In

contrast to the other amino acids, aspartate showed some tendency toward lower levels in the superficial molecular and granular layers than in deeper regions of the cochlear nucleus.

#### DISCUSSION

The prominent features of the amino acid distributions in rat, mentioned above, were similar to those in the cat (8), and the levels were also similar (Table II). Notably, the glycine levels were as high as those for cat, resembling the levels found by the same procedures in spinal cord ventral gray matter (3), where there is evidence for an inhibitory transmitter role of glycine (1, 4). The similarity in GABA levels between rat and cat contrasts with the reported 2.5-3 fold difference in the activity of the enzyme of GABA synthesis, glutamate decarboxylase (6).

One difference between the amino acid distributions in rat and cat particularly worth mentioning is that, except for the interstitial nucleus, the ventral cochlear nucleus in rat had relatively higher levels of the inhibitory amino acids GABA and glycine. Further, GABA levels were as high in the posteroventral as in the anteroventral cochlear nucleus of the rat, whereas the dorsal part of the anteroventral subdivision of cat had higher levels than either the ventral part or the posteroventral subdivision. These more uniform distributions of GABA and glycine in rat cochlear nucleus parallel similar trends for choline acetyltransferase and acetylcholinesterase (9, 10) and, to a lesser extent, for dry weight per volume.

In general, the dry weight per volume in the rat cochlear nucleus was very similar to that of the cat (Table II). The lower value in the acoustic striae of the rat may correlate with the more heterogeneous appearance of this region in thionin-stained sections of rat cochlear nuclei, in which neuronal somata are seen among the fiber bundles.

One implication from the data might be that the generally similar levels and distributions of putative transmitter amino acids in the cochlear nuclei of rats and cats are representative of similar levels and distributions in many mammals. Reported levels of GABA (as factor I) in bovine cochlear nucleus (7) and of GABA (6, 21 Table I) and aspartate and glutamate (23) in guinea pig cochlear nucleus further support this possibility.

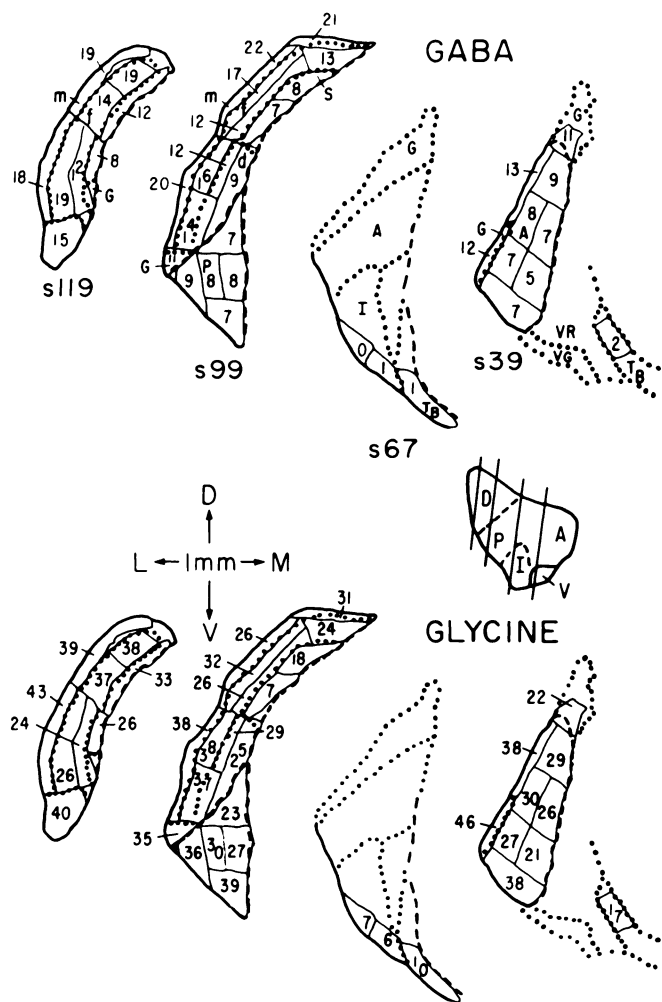


FIG. 3. Levels of GABA and glycine, in mmoles/kg dry weight, in transverse sections through the cochlear nucleus of rat B. Abbreviations and other details as in Figure 1.

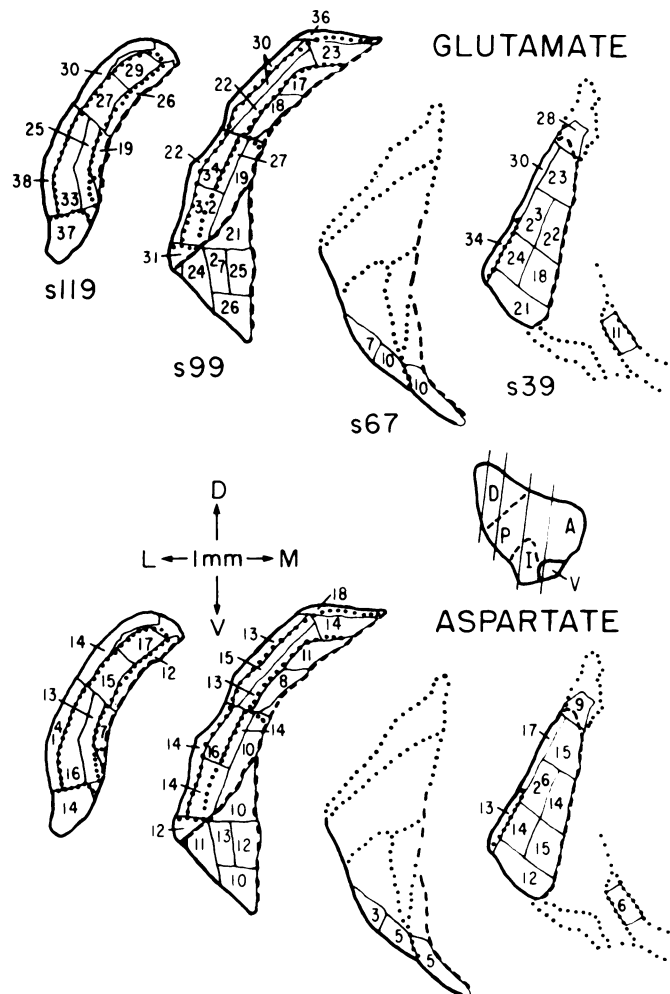


FIG. 4. Levels of glutamate and aspartate, in mmoles/kg dry weight, in the same transverse sections as in Figure 3, through the cochlear nucleus of rat B. Details as in Figure 1.

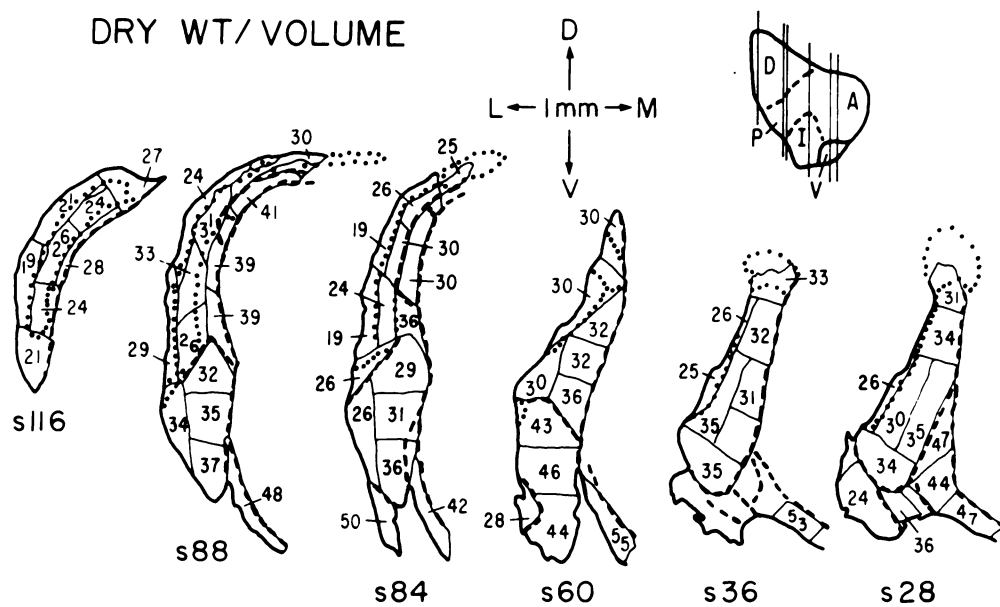


FIG. 5. Dry weight per volume, in kg/liter  $\times$  100, in the same transverse sections as in Figure 1, through the cochlear nucleus of rat A. Details as in Figure 1. Volume was measured as section thickness times the area determined on the section maps (9).

TABLE I  
Amino Acid Levels for Regions of Rat Cochlear Nuclei and Projection Tracts

Region <sup>a</sup>	Dry Wt <sup>b</sup> (kg/liter)	Amino Acid Level (mmoles/liter) Mean $\pm$ S.E.M. (No. of Samples)											
		GABA		Glycine		Glutamate		Aspartate					
		Rat A	B	A	B	A	B	A	B	A	B		
Granular region lateral to AVCN	0.30 $\pm$ 0.02 (5)	2.6 (2)	3.6 (1)	11.1 (1)	13.8 (1)	12.9 (1)	10.2 (1)	4.2 (1)	3.9 (1)				
DCN molecular layer	0.22 $\pm$ 0.01 (7)	3.1 $\pm$ 0.2 (7)	4.4 $\pm$ 0.2 (5)	8.1 $\pm$ 0.4 (7)	7.7 $\pm$ 0.7 (5)	10.1 $\pm$ 0.9 (5)	6.8 $\pm$ 0.7 (5)	2.6 $\pm$ 0.1 (5)	3.3 $\pm$ 0.2 (5)				
DCN fusiform-cell layer	0.27 $\pm$ 0.01 (7)	2.7 $\pm$ 0.1 (7)	4.1 $\pm$ 0.3 (8)	8.1 $\pm$ 1.1 (7)	8.4 $\pm$ 0.6 (8)	10.5 $\pm$ 1.1 (4)	7.6 $\pm$ 0.4 (8)	4.1 $\pm$ 0.2 (5)	4.1 $\pm$ 0.1 (8)				
DCN deep region	0.34 $\pm$ 0.02 (5)	1.9 (2)	3.6 (2)	5.8 (2)	9.2 (2)	6.8 (1)	7.8 (2)	3.1 (1)	4.1 (2)				
PVCN	0.35 $\pm$ 0.02 (5)	2.1 $\pm$ 0.2 (7)	2.8 $\pm$ 0.1 (5)	6.3 $\pm$ 0.4 (7)	10.9 $\pm$ 1.1 (5)	7.7 $\pm$ 1.1 (4)	8.8 $\pm$ 0.4 (5)	4.6 $\pm$ 0.7 (4)	3.9 $\pm$ 0.2 (5)				
AVCN	0.34 $\pm$ 0.01 (4)	2.0 $\pm$ 0.2 (8)	2.4 $\pm$ 0.2 (6)	9.5 $\pm$ 0.7 (8)	9.9 $\pm$ 1.0 (6)	8.5 $\pm$ 0.7 (4)	7.5 $\pm$ 0.3 (6)	6.1 $\pm$ 0.3 (4)	5.4 $\pm$ 0.7 (6)				
IN	0.50 $\pm$ 0.04 (4)	1.0 $\pm$ 0.3 (4)	0.3 (2)	3.5 $\pm$ 0.4 (4)	3.3 (2)	4.5 (1)	4.3 (2)	1.5 (1)	2.0 (2)				
Trapezoid body	0.49 $\pm$ 0.02 (9)	0.8 $\pm$ 0.2 (7)	0.7 (2)	3.9 $\pm$ 0.5 (7)	6.9 (2)	4.9 (2)	5.4 (2)	2.5 (2)	2.7 (2)				
Acoustic striae	0.35 $\pm$ 0.02 (5)	2.5 (2)	2.6 (2)	8.4 (2)	4.6 (2)	6.0 (1)	6.3 (2)	2.8 (1)	3.3 (2)				

<sup>a</sup> Abbreviations for Tables I and II are: AVCN, anteroventral cochlear nucleus; DCN, dorsal cochlear nucleus; IN, interstitial nucleus; PVCN, posteroventral cochlear nucleus.

<sup>b</sup> Mean  $\pm$  S.E.M. (number of sections measured). The values are based on measurements of sample volumes (area times thickness) in 12 sections, including those of Figure 5, of the right cochlear nucleus of rat A and 2 of rat B. The mean values and standard errors are based on the means for each section rather than the grand average of all samples because section thickness was the least accurate component of the measurement, and an inaccuracy in measuring the thickness of a given section would affect all samples in that section.

TABLE II  
Comparison of Amino Acid Levels in Regions of the Cochlear Nucleus and Projection Tracts of Rat and Cat<sup>a</sup>

Region	Dry Weight	GABA	Glycine	Glutamate	Aspartate
Granular region lateral to AVCN	1.1	1.0	1.6	1.1	1.2
DCN molecular layer	1.0	1.1	1.1	0.9	0.9
DCN fusiform-cell layer	1.0	0.8	0.9	0.9	0.7
DCN deep region	0.9	0.9	0.7	0.7	0.7
PVCN	0.9	1.8	1.3	0.8	0.8
AVCN	0.9	1.0	1.4	0.8	0.9
IN <sup>b</sup>	1.0	0.6	0.8	0.6	0.5
Trapezoid body	1.0	1.0	1.2	0.7	0.6
Acoustic striae	0.7	1.5	0.9	0.9	0.9

<sup>a</sup> The values are the ratios of the data, on a volume basis, for rat to those for cat from Table IV of Godfrey *et al.* (8). The data for rat are the averages of the values for rats A and B in Table I, except for GABA. Since there was a delay between sacrifice and freezing of the cat tissue, the GABA data for cat are compared to those of rat B.

<sup>b</sup> Since the region designated interstitial nucleus in the rat cochlear nucleus contained few nerve cell bodies, it is compared to the fibers-only part of the cat interstitial nucleus.

#### ACKNOWLEDGEMENTS

The authors are grateful to Jacquelyn Chapman for measuring sample volumes, to Anne Fuller Dillon for drawing the figures and to Linda Bird Hammond for typing the manuscript.

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