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QUANTITATIVE HISTOCHEMICAL MAPPING OF CANDIDATE TRANSMITTER AMINO ACIDS IN CAT COCHLEAR NUCLEUS[1]

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Levels of γ-amino-butyric acid, glycine, glutamate and aspartate were measured in samples dissected from freeze-dried sections of cat cochlear nuclei and the immediate surroundings. Refined methods of sampling and record-keeping were systematically applied at the histologic level. A detailed three-dimensional map of the distribution of the amino acids was obtained for one cochlear nucleus, and the results were compared with selected samples from five other nuclei. On a dry weight basis, γ-amino-butyric acid showed a 40-fold range, with highest levels in the molecular and fusiform cell layers of the dorsal cochlear nucleus. Glycine had a similar distribution as γ-amino-butyric acid although less striking. The aspartate maps differed from those of the other amino acids in that levels were lower in granular regions than in the ventral cochlear nucleus. The distribution of glutamate was least impressive and, on a volume basis, was almost uniform. The auditory nerve lacked high levels of any of the amino acids, as did the olivocochlear bundle, but some unusually high glycine levels were found in parts of the vestibular nerve. The highest γ-amino-butyric acid levels were in the lateral vestibular nucleus, where glutamate levels were remarkably low.

The study of transmitter systems in the cochlear nucleus is in a beginning stage. Some information has been gathered to suggest that acetylcholine is a transmitter at certain synapses (see reference 18). Gamma-amino butyric acid (GABA) has also been implicated as a transmitter (8, 9, 11a, 57, 62). However, little is known concerning other possible transmitters, or about which transmitters function at which synapses. Available evidence suggests that neither acetylcholine (38, 46) nor GABA (8, 9, 11a, 63) functions at the synapses of auditory-nerve fibers. Since these primary-fiber synapses have the appearance of chemical synapses, with synaptic vesicles (23, 30, 38), continued search for a chemical transmitter related to them is needed.

Our aim was to prepare precise maps of the distributions in the cochlear nucleus of four amino acids – GABA, glycine, glutamate and aspartate – since there is evidence that transmitters are especially concentrated in the neurons that release them (5, 27, 28, 35, 37, 60). Cats were used because anatomical and physiological data on the cochlear nucleus are most abundant for this animal (see references 4 and 25).

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METHODS

Data were obtained for cochlear nuclei from six cats sacrificed by various procedures (Table I). Since GABA levels have been reported to rise during postmortem storage of unfrozen brain tissue (21, 39), two special experiments were done. In the first experiment, cortical samples were obtained pre- and postmortem from the same cat (071274). In the second experiment, while a cat (102974) was under sodium pentobarbital (37 mg/kg intraperitoneal), the right cochlear nucleus was exposed by opening the skull and retracting the cerebellum, and was frozen *in situ* by pouring onto it Freon-12 chilled to its freezing point (−150°C) with liquid nitrogen. After about 150 ml of Freon had been poured, the animal was inverted and the entire head frozen in liquid nitrogen. After decapitation, in a room at −17°C, the head was kept very cold by periodic immersions in liquid nitrogen, and brain tissue blocks containing the individual cochlear nuclei were isolated with hacksaw and chisel. Some cochlear nuclear tissue, near the connection of the auditory nerve, was lost when the adjacent bone was cracked away.

Tissue blocks containing cochlear nuclei were mounted onto wooden dowels with brain paste (33) and stored at −80°C. Subsequently, 20 μm-thick sections were cut in a cryostat at −20°C. The proce-

TABLE I

Procedural Details on Isolation and Freezing of Cat Brain Tissue

Cat Brain Tissue Block No.[a]	Method of Sacrifice	Postmortem Time (Min) Until Freezing of Tissue	Method of Freezing Tissue
021673 L,R	Overdose of sodium pentobarbital	50	Dropped into Freon
012674 L,R	Decapitated while anesthetized with ether	50	Dropped into Freon
022374, L,R	Injection of about 50 cc of air into the heart	60	Dropped into Freon
071274 L,R	Decapitated while anesthetized with sodium pentobarbital (37 mg/kg i.p.)	(L)70,(R)60	Dropped into Freon
102974 L,R	Freezing of entire head while anesthetized with sodium pentobarbital (37 mg/kg i.p.)	(L)0-5,(R)0	(L)Immersed in liquid nitrogen *in situ* (R)Superfused with Freon *in situ*
111374 L,R	Freezing of entire head while anesthetized with diallybarbituric acid (75 mg/kg i.p.)	0-5	Immersed in liquid nitrogen *in situ*

[a] Each tissue block is denoted by the cat number, indicating the date of sacrifice, followed by the letter representing the left (L) or right (R) side of the brain. Cats were used directly as obtained from a supplier with two exceptions: cat 021673 was obtained from the laboratory of Dr. C. Hunt, where it had been used several hours for an experiment on the tail, under sodium pentobarbital anestheisa. Cat 111374 was obtained from the laboratory of Dr. R. R. Pfeiffer, where it had been used for a 2-day experiment during which recordings were made from the left auditory nerve with procedures similar to those described by Kiang et al. (26). Sounds were presented to the left ear via a closed acoustic system, while the cat, anesthesized with diallylbarbituric acid (75 mg/kg), was maintained in a sound-proof chamber.

dures for cutting sections and saving them in order have been described (17). Sections were freeze-dried (33), then stored under vacuum at −23°C. Dissections were done at room temperature, utilizing nearby thionin-stained freeze-dried sections as guides (17, 32). An exact record (17) of the location of each piece analyzed permitted maps to be constructed showing the distributions of the amino acids. The pieces of tissue were weighed (0.2–2 μg) on a quartz fiber balance (33); volumes (0.5–5 nl) were calculated as thickness times the area measured on the maps (17). Since the dry weight could be measured more reliably than the volume, amino acid levels to be presented for individual samples will be referenced to dry weight. However, average values for regions will be referenced to volume as well as dry weight, since, for the cochlear nucleus (17) as well as other brain regions (34), the volume of a sample relates closely to the lipid-free fraction, which would be expected to contain the bulk of the amino acids.

Each sample piece was loaded into a 1 μl droplet of 0.05 N NaOH under oil in an oil well (36). After heating at 80°C for 20 min, four 0.2-μl aliquots were taken from each droplet into four separate oil well racks for measurement of GABA, glycine, glutamate and aspartate content. The assay procedures are described elsewhere (2).

RESULTS

Postmortem changes and inter-animal consistency of distribution patterns: In frontal cortex, GABA rose more than 2-fold during 40 min of total ischemia, glycine increased about 25%, while glutamate and aspartate changed little if at all (Table II).

On the other hand, if similar postmortem changes occur in the cochlear nucleus, they were obscured by differences from one animal to another. Figure 1 illustrates the approach used to compare data for similar regions of different cochlear nuclei. Data from such comparisons are summarized in Table III. Comparison between a cochlear nucleus frozen *in situ* from one cat with those frozen about an hour postmortem from two other cats (Table III, first five columns) indicates that GABA levels may have risen only slightly during this period of prolonged ischemia, while glycine levels may have fallen and aspartate levels risen somewhat. Despite individual differences in absolute levels, the patterns of distribution among the regions were similar for the three cats.

Amino acid levels were similar in most cases for the various cochlear nuclei from animals killed with or without brief anesthesia or after long anesthesia (Table III). Two notable exceptions were lower aspartate levels in the dorsal cochlear nucleus of the animal maintained for several hours under sodium pentobarbital, and much lower GABA levels for the animal maintained for 2 days under Dial-urethane.

TABLE II

Postmortem Changes of Amino Acid Levels in Cat Frontal Cortex[a]

	Frozen Immediately	Frozen 40 Min Postmortem
	mmoles/kg wet wt[b]	
GABA	0.76	1.81
Glycine	0.70	0.87
glutamate	9.1	9.5
aspartate	2.40	2.45

[a] Cat 071274 was anesthetized with sodium pentobarbital (37 mg/kg intraperitoneal) and a 150 mg piece of right frontal cortex excised and immediately frozen in Freon-12 chilled to its freezing point with liquid nitrogen. The head was then cut off and, after 40 min at room temperature, a corresponding 150 mg piece of left frontal cortex was excised and frozen.

[b] The data may be converted to a volume basis by multiplying by 1.05 (32).

Amino acid distribution patterns in the cochlear nucleus: Of the various cochlear nuclei sampled, one was chosen for a more complete mapping study because the animal received no anesthesia and because the angle of sectioning was favorable for comparison with a cochlear nucleus block model (25).

The most striking distribution pattern was that of GABA (Fig. 2). The molecular and fusiform cell layers of the dorsal cochlear nucleus contained the highest levels, about 40-fold higher, on a dry weight basis, than levels in parts of the interstitial nucleus, and about 7 times those in the immediately frozen cortex piece. There were gradients of GABA levels in the anteroventral cochlear nucleus, with higher levels in the more dorsal and rostral parts, and in the deep region of the dorsal

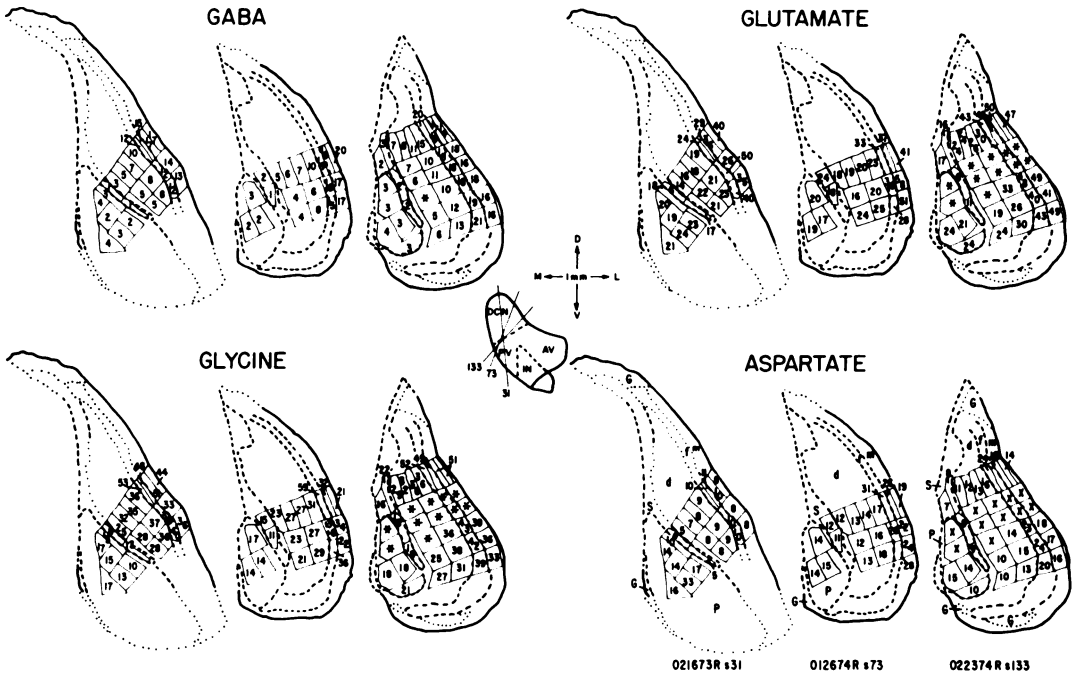


FIG. 1. Comparison of amino acid distributions for similar regions of cochlear nuclei from three different cats. Amino acid levels are in mmoles/kg dry weight. Asterisks indicate samples for which data were not obtained. Tissue block and section numbers are given at the lower right, and approximate locations of the transverse sections are projected onto the side-view drawing of the cochlear nucleus as described elsewhere (17). The directional calibration scale pertains to the sections. Within the sections, thin lines are the boundaries of excised sample pieces, while thick lines are regional boundaries. Regional boundaries marked by dashed lines are more approximate than those marked by solid lines, while dotted lines are based on reference to nearby thionin-stained sections.

TABLE III

Amino Acid Levels for Comparable Locations in Some Regions of Cat Cochlear Nuclei (mmoles/kg dry wt). Mean ± SEM (No. of Samples)[a, b]

Region	CAT BRAIN TISSUE BLOCK NO. AND SPECIAL CONDITIONS					
	102974R Frozen *in situ*	022374L No Anesthesia	022374R No Anesthesia	012674R Brief Anesthesia	021673R Anesthetized Several Hr	111374R Anesthetized 2 Days
	Rostral	Rostral Caudal	Caudal	Caudal	Caudal	Caudal
GABA						
DCN molecular layer	17 ± 1.0(8)	21 ± 0.8(7), 18 ± 0.8(13)	19 ± 0.9(12)	18 ± 0.8(4)	15 ± 1.0(4)	7.0 ± 1.2(6)
DCN fusiform cell layer	16 ± 0.9(8)	20 ± 0.8(7), 19 ± 0.6(10)	21 ± 0.8(12)	17 ± 1.2(3)	13 ± 0.8(4)	8.3 ± 1.8(6)
DCN deep region	8.3 ± 0.6(11)	10 ± 0.9(13), 9.1 ± 0.9(19)	9.6 ± 0.6(27)	6.3 ± 0.7(8)	6.4 ± 0.8(8)	2.3 ± 0.4(12)
PVCN	3.3 ± 0.3(14)	3.5 ± 0.3(37), 3.7 ± 0.2(20)	4.3 ± 0.7(8)	2.3 ± 0.3(3)	3.0 ± 0.4(6)	1.5 ± 0.3(4)
Acoustic striae		3.0 ± 0.3(5)	2.6 ± 0.3(8)	1.5(2)	1.0(2)	0.4 ± 0.03(3)
Glycine						
DCN molecular layer	34 ± 3.0(4)	33 ± 2.2(7), 36 ± 1.3(13)	36 ± 2.8(10)	27 ± 3.4(4)	41 ± 4.1(4)	36 ± 8.0(6)
DCN fusiform cell layer	52 ± 2.3(4)	34 ± 1.3(7), 36 ± 1.7(9)	39 ± 2.4(10)	44 ± 7.7(3)	45 ± 3.5(4)	48 ± 6.3(6)
DCN deep region	37 ± 1.0(4)	27 ± 1.1(13), 25 ± 1.2(19)	27 ± 1.3(23)	26 ± 1.2(8)	32 ± 1.6(8)	23 ± 2.3(12)
PVCN	27 ± 2.2(13)	16 ± 0.5(37), 16 ± 0.8(20)	16 ± 1.4(6)	15 ± 1.0(3)	14 ± 1.1(6)	15 ± 0.9(4)
Acoustic striae		14 ± 1.1(5)	15 ± 1.7(8)	13(2)	11(2)	12 ± 2.6(3)
Glutamate						
DCN molecular layer	48 ± 1.5(8)	44 ± 1.7(7), 43 ± 1.2(9)	47 ± 1.6(5)	47 ± 4.8(4)	34 ± 3.0(4)	47 ± 2.6(6)
DCN fusiform cell layer	37 ± 2.2(8)	39 ± 1.9(7), 39 ± 1.2(8)	44 ± 1.6(5)	32 ± 2.3(3)	36 ± 5.8(4)	40 ± 2.6(6)
DCN deep region	24 ± 0.9(10)	25 ± 1.1(13), 25 ± 1.1(13)	26 ± 1.4(9)	21 ± 1.1(8)	19 ± 1.1(8)	21 ± 1.4(12)
PVCN	27 ± 0.8(13)	26 ± 0.6(37), 24 ± 0.8(10)	23 ± 1.0(3)	19 ± 0.9(3)	21 ± 0.9(6)	20 ± 1.8(4)
Acoustic striae		18 ± 0.6(4)	13 ± 2.5(4)	21(2)	12(2)	12 ± 0.7(3)
Aspartate						
DCN molecular layer	11 ± 0.8(8)	15 ± 1.7(7), 16 ± 0.9(13)	17 ± 0.7(5)	23 ± 1.5(4)	8.5 ± 0.5(4)	16 ± 2.3(6)
DCN fusiform cell layer	15 ± 0.8(8)	22 ± 1.7(7), 19 ± 0.5(9)	23 ± 0.8(5)	28 ± 1.5(3)	10 ± 0.3(4)	24 ± 1.2(6)
DCN deep region	9.6 ± 0.4(11)	12 ± 1.0(13), 12 ± 0.5(19)	13 ± 0.7(9)	14 ± 0.8(8)	7.9 ± 0.5(8)	10 ± 0.9(11)
PVCN	12 ± 0.5(14)	14 ± 0.4(37), 15 ± 0.4(20)	13 ± 1.5(3)	14 ± 0.3(3)	17 ± 3.3(6)	11 ± 0.9(4)
Acoustic striae		8.4 ± 1.2(5)	8.0 ± 0.4(4)	12(2)	4.5(2)	6.3 ± 1.2(3)

[a] The data of columns 4-6 are those shown in Figure 1, plus additional GABA and glycine data for comparable locations in section 129 of tissue block 022374R. The data for tissue block 022374L are from sections 43 and 47 (caudal) and section 91 (1 mm farther rostral) (Figs. 2-5). The data of column 1 are from rostral locations, whereas those of columns 4-7 are from caudal locations. Since the "rostral" and "caudal" data of tissue block 022374L are comparable, the data of column 1 can probably be compared without much error with those of all other columns.

[b] The abbreviations used in Tables III and IV are: DCN, dorsal cochlear nucleus; PVCN, posteroventral cochlear nucleus; IN, interstitial nucleus; AVCN, anteroventral cochlear nucleus.

cochlear nucleus, with higher levels in the more superficial parts.

The distribution of glycine (Fig. 3) tended to parallel that of GABA, with highest levels in granular regions and the dorsal cochlear nucleus, but almost as high levels in the ventral cochlear nucleus. Although the distribution of this amino acid was less striking than that of GABA (an 8-fold range on a dry weight basis), the highest levels were more than an order of magnitude higher than those in the frontal cortex. For both glycine and GABA, levels were higher in the acoustic striae than in the interstitial nucleus or trapezoid body.

The distribution of glutamate (Fig. 4) generally paralleled that of the nonlipid fraction of the dry weight (17). When the data were expressed on the basis of volume (Fig. 6), the distribution looked rather even. The levels were similar to those of the frontal cortex.

Aspartate (Fig. 5) differed from the other amino acids in that levels were lower in the granular regions than in the ventral cochlear nucleus. This trend was more pronounced when data were expressed on a volume basis (Fig. 6), in which case also levels in the dorsal cochlear nucleus, except within the fusiform cell layer, were lower than those in the ventral cochlear nucleus. The highest aspartate levels were about 3 times those in the frontal cortex.

Distribution of amino acids and choline acetyltransferase in the vestibular nerve: The vestibular nerve contains olivocochlear (49, 50) and centrifugal vestibular fibers (13) directed toward the cochlear and vestibular sensory structures, respectively. Since a branch of the olivocochlear bundle projects to the cochlear nucleus (50), the possible association of any of the amino acids with these centrifugal fibers was of interest here (Fig. 7). The centrifugal fibers in the vestibular nerve have been previously visualized by staining for acetylcholinesterase (15, 46, 51, 52, 54–56), so that an adjacent section stained for cholinesterase can be used to indicate their localization. For comparison with the amino acid levels, choline acetyltransferase activities were measured in another nearby section. Although choline acetyltransferase activities were preferentially high in the parts of the vestibular nerve where cholinesterase-positive fiber bundles were seen, amino acid levels were not. The amino acid levels were generally higher in the nerve root than in the peripheral nerve. Glycine levels were noticeably elevated in some samples near the ganglia in the nerve. The average levels of glycine in the vestibular nerve and its root were higher than those for most other nerves studied (Table IV), while the highest glycine levels were about half as high on a volume basis as any in the cochlear nucleus.

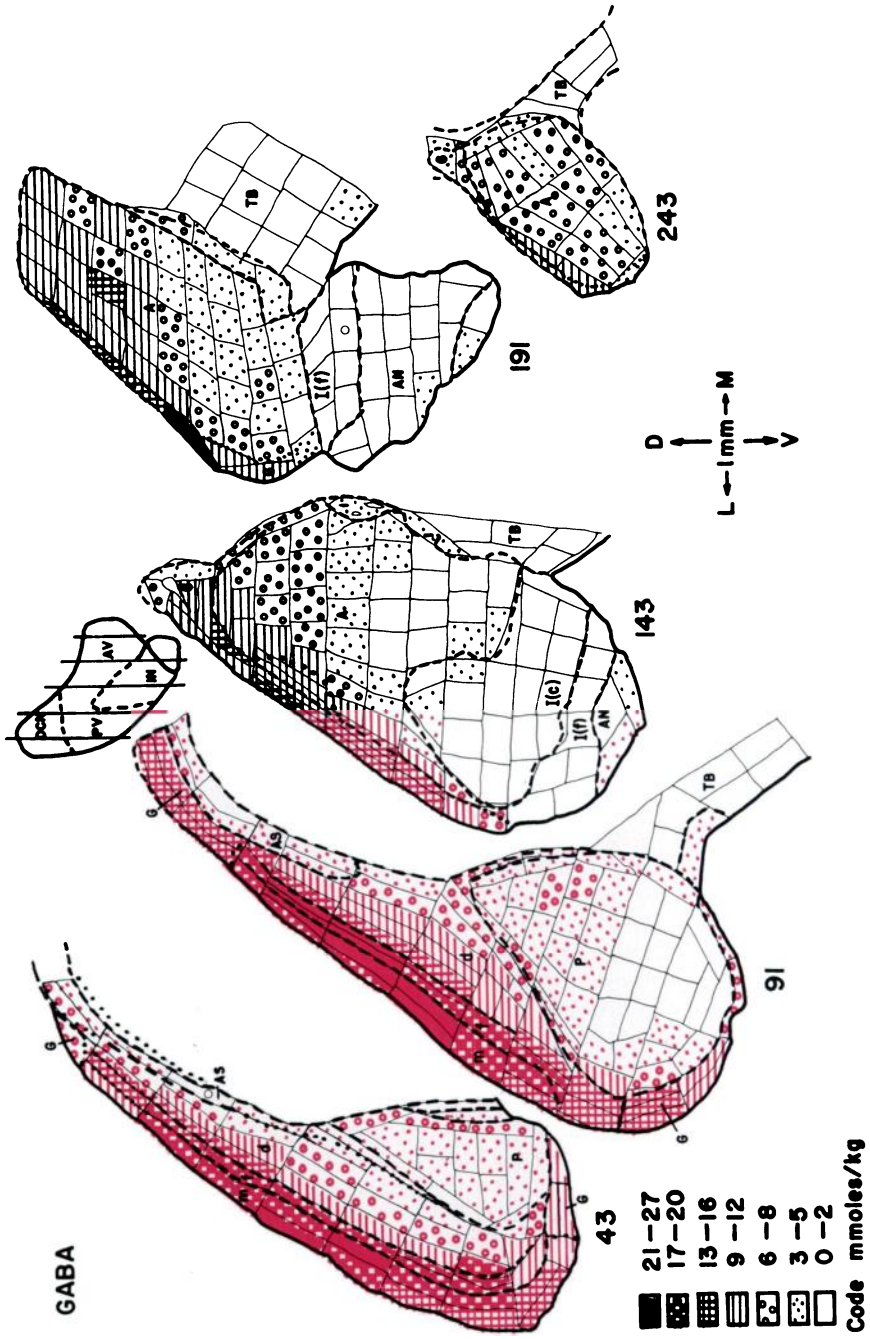
Amino acid levels in other regions near the cochlear nucleus: For comparison with the amino acid levels in the cochlear nucleus, a few measurements were made in nearby regions of the brain stem and cerebellum (Table IV).

Except for the vestibular nerve ganglion, the other gray matter regions examined had GABA concentrations as high as any layer of the dorsal cochlear nucleus. GABA concentrations were highest in the dorsal division of the lateral vestibular nucleus, but these were only about $^3/_2$ those in the fusiform cell layer of the dorsal cochlear nucleus. Glycine concentrations as high as those in most regions of the cochlear nucleus were found only in the facial nucleus and spinal trigeminal nucleus. Glutamate concentrations varied little among gray matter regions, except for remarkably low concentrations in the dorsal division of the lateral vestibular nucleus and in the vestibular nerve ganglion. The 3-fold range of aspartate concentrations among the other brain regions was similar to that among the various regions of the cochlear nucleus.

There were considerable ranges of amino acid concentrations among regions of white matter: 7-fold for GABA, 4.5-fold for glycine, 2.5-fold for glutamate and 3-fold for aspartate. None of the non-auditory tracts had as high glycine levels as the acoustic striae, or as high aspartate levels as the trapezoid body. The concentrations in the facial nerve root might be taken as representative of a tract where the amino acids would serve no transmitter role, since this region should consist of cholinergic motor axons (see reference 18). The GABA concentration in the cerebellar white matter may be representative of a tract containing some GABA-ergic axons, since it contains the axons of the Purkinje cells, which probably release GABA as a transmitter (42).

DISCUSSION

Comparison with other studies: The GABA level found here for the cochlear nucleus as a


GABA
43
91
143
191
243
TB
AN
I(f)
I(c)
AV
IM
PV
P
G
D
L←1mm→M
V
Code mmoles/kg
0–2
3–5
6–8
9–12
13–16
17–20
21–27

whole (calculated as in Table V of reference 18) is about 2/3 that reported by Tachibana and Kuriyama (57), but 1.7 times the value reported by Fisher and Davies (11a), both for guinea pig cochlear nucleus. The distribution of GABA within the nucleus seen in the present study agrees with those reports in that GABA levels tend to be higher in the dorsal parts of the nucleus. However, the GABA levels in the map presented by Tachibana and Kuriyama are about five times those reported here, and do not agree with their own average value for the whole nucleus. If Florey and Florey's (12) factor I measurements are converted to equivalent GABA levels using their conversion factor, then the level reported for bovine dorsal cochlear nucleus is about 2/3 of what we found, although it closely resembles our value for whole cochlear nucleus. (However, it would require a special effort to isolate only the dorsal cochlear nucleus from a fresh brain.) We are not aware of any previously reported glycine, glutamate or aspartate levels in the cochlear nucleus.

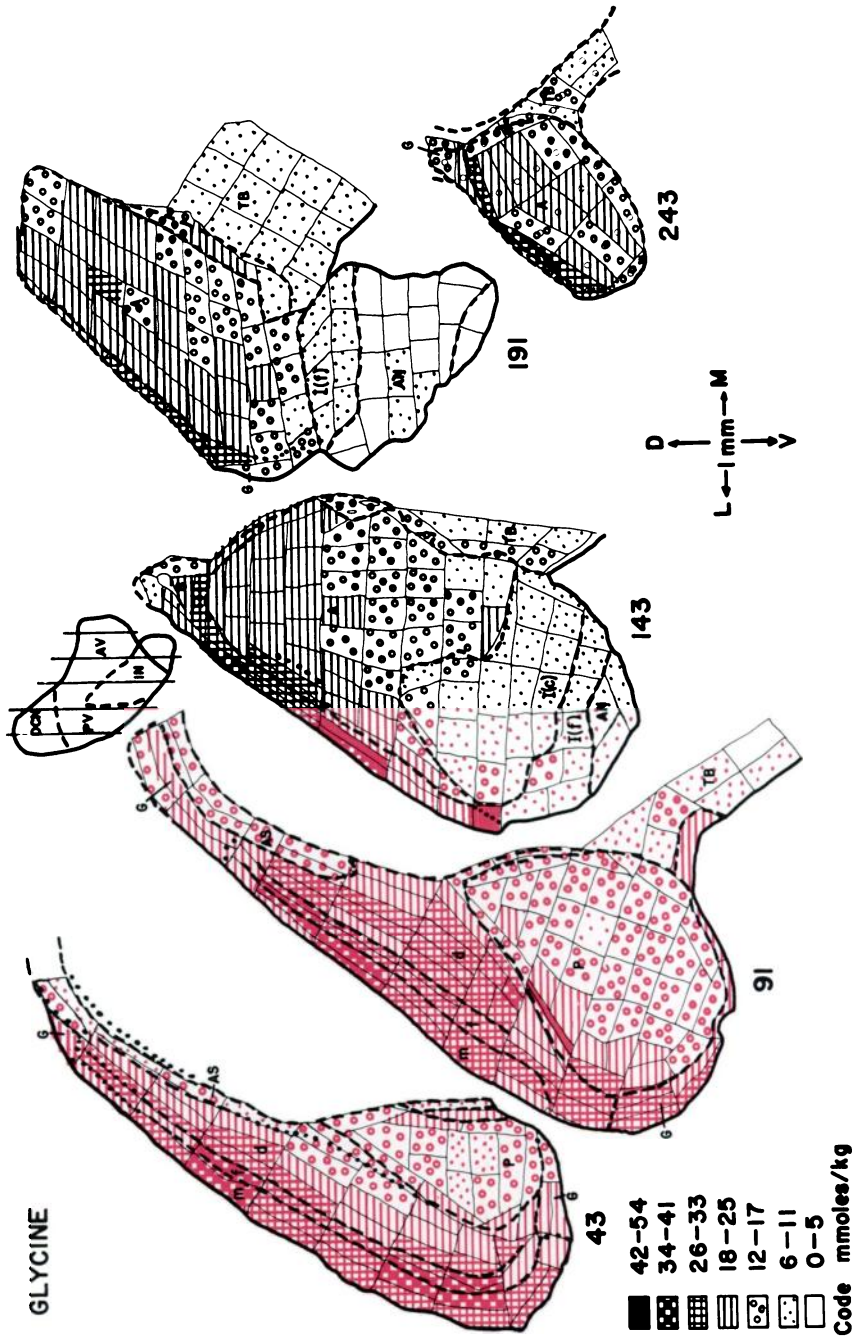
Concerning the other brain stem regions, the value for GABA in the restiform body is about as low as the equivalent value of Florey and Florey (12) for the bovine restiform body, while our value for GABA in the dorsal division of the lateral vestibular nucleus resembles the level reported for dorsal Deiters' cells by Otsuka *et al.* (47).

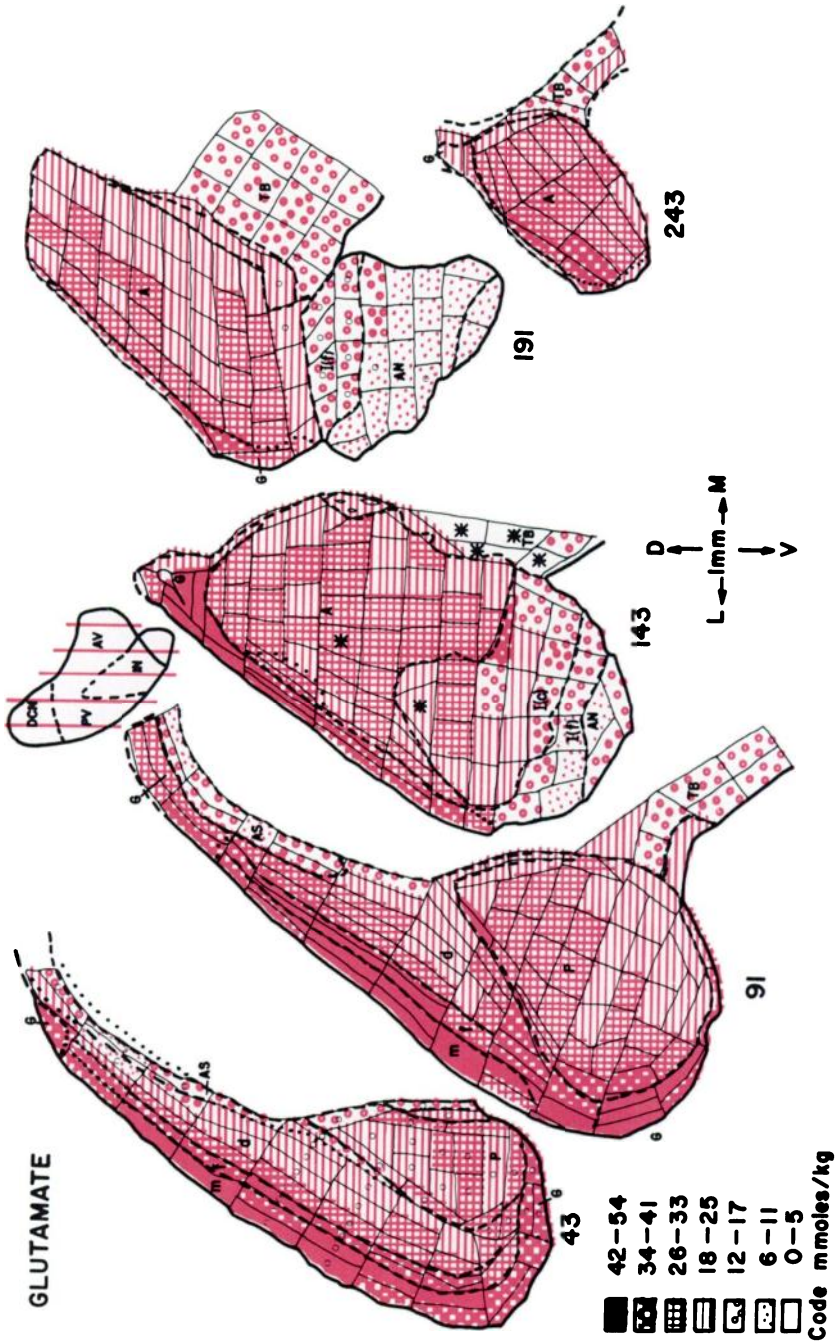
The GABA levels found in cerebellar layers resemble those reported previously for other species (21, 29), except that the values for white matter are somewhat higher, and we were unable to find higher levels for samples of the Purkinje cell layer than for the other layers. Such differences could easily relate to the fact that these samples were from the flocculus of the cat, while those of other authors were from other species and were probably from more accessible lobes. The results of Florey and Florey (12) suggest that there may be some variation in GABA levels among cerebellar lobes.

Possible relations of amino acids to synaptic transmission in the cochlear nucleus: A primary question about synaptic transmission in the cochlear nucleus concerns the substance released by auditory nerve terminals. None of the amino acids had high levels in the auditory nerve, but those of aspartate were somewhat higher than in most other white matter studied. When the distributions of the amino acids in the cochlear nucleus were examined, only that of aspartate showed some similarities to the reported distribution of auditory nerve terminals (6, 31, 38, 44, 48, 52), in that levels were lower in granular regions and, on a volume basis, in the molecular layer of the dorsal cochlear nucleus than in the ventral cochlear nucleus. The distributions of aspartate in the cochlear nucleus and in the cochlea (16, 58) suggest that further studies on its relation to auditory nerve fibers might prove fruitful. It has been suggested that some of the synapses of auditory nerve fibers onto octopus cells in the posteroventral cochlear nucleus may be inhibitory (22, 41). If so, the unimpressive levels of GABA and glycine in the auditory nerve argue against either of these amino acids being the inhibitory transmitter.

The lack of evidence for associating GABA or glycine with the synapses of auditory nerve fibers implies that the high levels of these

FIG. 2–5. The distributions of the amino acids within the cochlear nucleus of tissue block 022374L. Amino acid levels are coded as indicated. The approximate locations of the transverse sections are shown, in order, in the side view drawing of the cochlear nucleus. Boundaries and calibration are as in Figure 1. Asterisks indicate samples for which data are not available; these pieces have been coded so as to fit with their surroundings. Small circles within pieces indicate that the data have been obtained from a nearby section dissected with sample locations made as close as possible to those shown. The nearby sections used for this purpose were numbers 47, 195, and 239. The sections correspond to those of the cochlear nucleus block model (25) as follows: 43 with T-11, 91 with T-22, 143 with T-34, 191 with T-44 and 243 with T-58. The sections can also be compared with the stained sections shown by Brawer *et al.* (4) by noting that their section 110 is the basis for section T-5 of the block model, 122 for T-8, etc. The abbreviations used in Figures 1–7 are: A, AV, AVCN, anteroventral cochlear nucleus; AN, auditory nerve; AS, acoustic striae; CbF, cerebellar flocculus; DCN, dorsal cochlear nucleus; m, molecular layer of dorsal cochlear nucleus; f, fusiform cell layer of dorsal cochlear nucleus; d, deep (polymorphic) layer of dorsal cochlear nucleus; FN, facial nerve; G, granular region of cochlear nucleus; GABA, γ-amino butyric acid; I, IN, interstitial nucleus; I(c), part of interstitial nucleus containing nerve cell bodies; I(f), part of interstitial nucleus containing primarily nerve fibers and few if any neuronal somata; P, PV, PVCN, posteroventral cochlear nucleus; TB, trapezoid body; TNR, spinal trigeminal tract (descending trigeminal nerve root); VN, VNR, vestibular nerve, root.


GLYCINE
43
91
143
191
243
D
L ← 1 mm → M
V
Code mmoles/kg
42-54
34-41
26-33
18-25
12-17
6-11
0-5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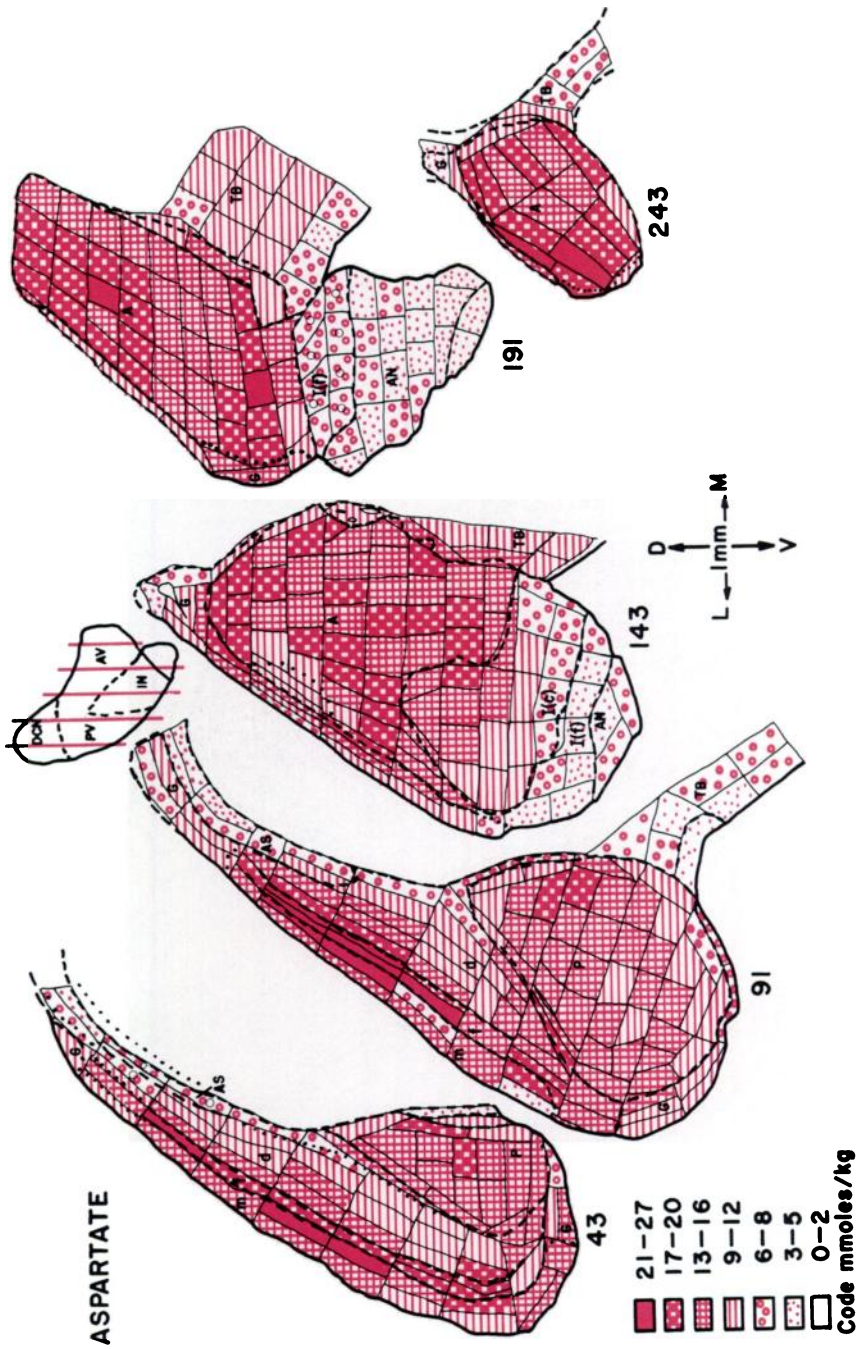
ASPARTATE
43
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9–12
13–16
17–20
21–27

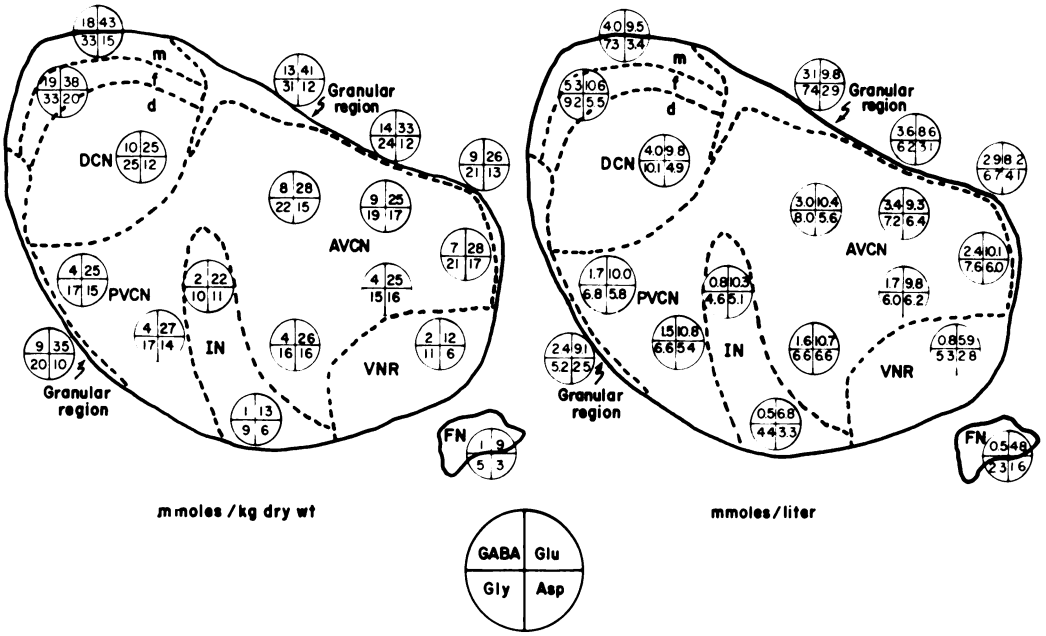

FIG. 6. Average levels of amino acids for regions of the cochlear nucleus of tissue block 022374L, based on dry weight and on volume. The sagittal section with boundaries was traced from section 368P of Brawer *et al.* (4). Glu, glutamate; Gly, glycine; Asp, aspartate.

amino acids found within the cochlear nucleus might relate to a role in other pathways to the nucleus or in interneurons. Our data offer clues on certain points: (*a*) Elevated GABA, and especially high glycine levels in the acoustic striae might be associated with some pathways leaving (11, 31, 45, 61) or entering (14, 20, 50, 59) the nucleus via this route; (*b*) The levels of GABA in the superficial layers of the dorsal cochlear nucleus approximate the levels in the spinal cord dorsal horn (2, 19, 40), cerebellar cortex and lateral vestibular nucleus, where there is strong evidence for a transmitter role (7, 53). These elevated GABA levels and those in the dorsal part of the anteroventral cochlear nucleus might relate to reciprocal axonal connections between the anteroventral and dorsal subdivisions of the cochlear nucleus (31), especially since the anteroventral-to-dorsal component seems to be inhibitory (10). In agreement with such a proposal, Davies (9) has found GABA transaminase staining of fusiform cells in the dorsal cochlear nucleus and of many cells in the anteroventral cochlear nucleus, suggesting that these neurons might be related to synapses where GABA is released. However, this proposal is not entirely consistent with the anatomical findings of Lorente de Nó (31), who showed layers 3 and 4 (the deep region) of the dorsal cochlear nucleus connected to the anteroventral cochlear nucleus, whereas we found the highest GABA levels in layers 1 (molecular), 2 (fusiform cell) and 3; (*c*) Glycine levels in the cochlear nucleus are similar to those reported for spinal cord gray matter (2, 19, 40), where there is much evidence for an inhibitory transmitter role of glycine in interneurons (1, 7). Perhaps glycine is associated with some of the many small cells scattered throughout most of the cochlear nucleus (43), many of which, especially in the dorsal cochlear nucleus, seem to be interneurons (24, 31, 45). A problem with relating all the glycine to small cells is that glycine levels are high in the octopus cell region in the caudal part of the posteroventral cochlear nucleus, where there are said to be very few small cells (22, 43).

A major value of the maps presented here is to serve as references for future work on the cochlear nucleus. Physiologic iontophoretic injection studies could use the maps as guides to the most promising regions for testing the effects of particular amino acids or related drugs. Also, changes in the distributions of the amino

TABLE IV

Amino Acid Levels for Regions of a Cat Brain[a]

Region[b]	Dry Wt[c]	Amino Acid Level (mmoles/liter) Mean ± SEM (No. of Samples)			
		GABA	Glycine	Glutamate	Aspartate
	kg/liter				
Granular region dorsomedial to DCN	0.26 ± 0.02(7)	3.4 ± 0.04(5)	4.7 ± 0.3(5)	8.6 ± 0.5(5)	2.4 ± 0.1(5)
Granular region ventral to PVCN	0.26 ± 0.03(6)	2.4 ± 0.5(5)	5.2 ± 0.3(5)	9.1 ± 0.4(5)	2.5 ± 0.4(5)
Granular region lateral to AVCN	0.28 ± 0.02(12)	3.5 ± 0.2(19)	7.8 ± 0.6(17)	10.1 ± 0.5(19)	3.4 ± 0.2(19)
DCN molecular layer	0.22 ± 0.01(9)	4.0 ± 0.2(26)	7.3 ± 0.3(26)	9.5 ± 0.2(22)	3.4 ± 0.1(26)
DCN fusiform cell layer	0.28 ± 0.01(9)	5.3 ± 0.2(21)	9.2 ± 0.4(20)	10.6 ± 0.3(19)	5.5 ± 0.2(20)
DCN deep region	0.40 ± 0.01(8)	4.0 ± 0.2 (38)	10.1 ± 0.3(38)	9.8 ± 0.3(31)	4.9 ± 0.2(37)
PVCN caudal part	0.40 ± 0.01(4)	1.7 ± 0.1(26)	6.8 ± 0.3(26)	10.0 ± 0.3(13)	5.8 ± 0.2(26)
PVCN rostral part	0.40 ± 0.01(3)	1.5 ± 0.1(41)	6.6 ± 0.3(41)	10.8 ± 0.3(41)	5.4 ± 0.2(41)
IN part containing neuron somata	0.47 ± 0.04(4)	0.8 ± 0.1(20)	4.6 ± 0.2(20)	10.3 ± 0.7(19)	5.1 ± 0.3(20)
AVCN caudoventral part	0.41 ± 0.02(7)	1.7 ± 0.1(51)	6.5 ± 0.2(51)	10.7 ± 0.4(48)	6.6 ± 0.1(51)
AVCN caudodorsal part	0.38 ± 0.02(7)	3.3 ± 0.1(38)	7.6 ± 0.2(38)	9.8 ± 0.2(38)	6.2 ± 0.1(38)
AVCN rostral part	0.36 ± 0.01(6)	2.4 ± 0.1(30)	7.6 ± 0.5(15)	10.1 ± 0.3(30)	6.0 ± 0.2(30)
IN part containing only fibers	0.51 ± 0.03(7)	0.5 ± 0.1(23)	4.4 ± 0.2(24)	6.8 ± 0.3(17)	3.3 ± 0.2(18)
Auditory nerve	0.51 ± 0.03(6)	0.8 ± 0.1(24)	2.7 ± 0.1(24)	4.9 ± 0.2(22)	2.8 ± 0.1(24)
Trapezoid body	0.50 ± 0.02(15)	0.7 ± 0.1(33)	4.6 ± 0.2(29)	7.6 ± 0.2(28)	4.1 ± 0.2(33)
Acoustic striae	0.52 ± 0.02(6)	1.7 ± 0.1(21)	7.3 ± 0.3(22)	7.2 ± 0.4(18)	3.4 ± 0.2(20)
Cerebellar flocculus molecular layer	0.26 ± 0.02(6)	5.0 ± 0.1(9)	3.3 ± 0.3(10)	10.7 ± 0.6(10)	2.6 ± 0.2(10)
Cerebellar flocculus Purkinje-cell layer	0.26 ± 0.03(4)	4.9 ± 0.3(3)	3.6 ± 1.6(3)	10.9 ± 0.1(3)	3.4 ± 0.2(3)
Cerebellar flocculus granular layer	0.29 ± 0.03(6)	4.8 ± 0.3(7)	3.8 ± 0.3(8)	13.3 ± 1.2(8)	3.7 ± 0.4(8)
Infracerebellar nucleus	0.32(2)	7.0 ± 0.6(4)	5.9 ± 0.3(4)	9.4 ± 0.9(4)	5.1 ± 0.3(4)
Lateral vestibular nucleus, dorsal division	0.36 ± 0.01(4)	8.0 ± 0.4(9)	5.3 ± 0.2(9)	4.7 ± 0.2(9)	4.3 ± 0.4(9)
Spinal trigeminal nucleus	0.36 ± 0.02(7)	4.1 ± 1.1(4)	8.3 ± 0.8(4)	9.4 ± 0.4(4)	5.4 ± 0.5(4)
Facial nucleus	0.39 ± 0.05(3)	5.5(2)	9.6(2)	11.3(2)	7.2(2)
Vestibular nerve ganglion	0.28(2)	0.6 ± 0.1(4)	3.2(2)	3.9 ± 0.3(4)	2.3 ± 0.3(3)
Cerebellar flocculus white matter	0.43 ± 0.02(7)	3.4 ± 0.3(9)	3.9 ± 0.4(9)	7.7 ± 0.5(9)	1.6 ± 0.2(9)
Restiform body	0.47 ± 0.01(4)	0.5 ± 0.1(8)	1.6 ± 0.2(8)	8.3 ± 0.3(8)	1.9 ± 0.1(8)
Spinal trigeminal tract	0.43 ± 0.01(11)	1.4 ± 0.2(4)	3.8 ± 0.5(4)	7.1 ± 0.4(4)	2.2 ± 0.2(3)
Facial nerve root	0.46 ± 0.03(8)	0.9(2)	3.2(2)	4.6(2)	1.8(2)
Facial nerve	0.51 ± 0.02(5)	0.5 ± 0.2(4)	2.3(2)	4.7 ± 0.4(3)	1.5 ± 0.00(4)
Vestibular nerve root	0.49 ± 0.02(6)	0.8 ± 0.1(10)	5.3 ± 0.2(7)	5.9 ± 0.3(8)	2.8 ± 0.1(10)
Vestibular nerve	0.47 ± 0.02(4)	0.5 ± 0.04(14)	4.7 ± 0.4(11)	3.6 ± 0.2(14)	1.8 ± 0.1(13)

[a] The data are for cat brain tissue block 022374L. They are grouped into gray matter and white matter of the auditory regions, then gray and white of other regions.

[b] Regional names, other than for the cochlear nucleus, are based on Berman (3). The amino acid levels for cochlear nucleus regions were averaged from the sections shown in Figures 2-5 as well as other nearby sections. The levels for other regions are based on samples obtained from within those regions.

[c] Mean ± SEM (number of sections measured). The values are based on measurements of sample volumes (area times thickness) in 12 sections of tissue block 012674L, 4 of tissue block 012674R, 6 of tissue block 022374L, 1 of tissue block 022374R, and 5 of tissue block 011273L. The mean values and standard errors in the table are based on the means for each section rather than the grand average of all samples because an inaccuracy in the measurement of the thickness of a given section would affect all samples in that section.

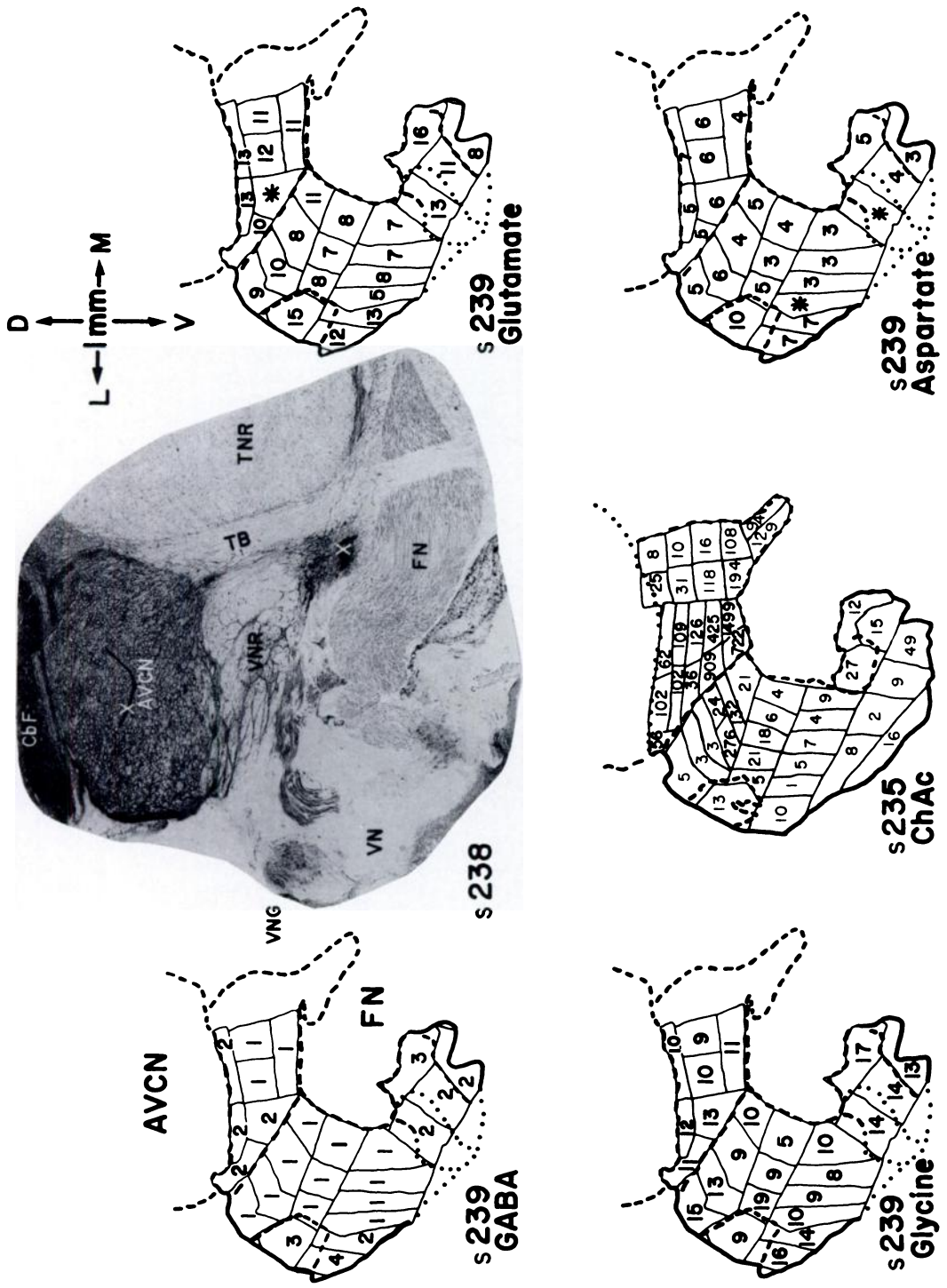

FIG. 7. Distribution of amino acids and enzymes of the cholinergic system in transverse sections of the vestibular nerve and root. Amino acid levels are expressed as mmoles/kg dry weight, choline acetyltransferase activities as μmoles/kg dry weight/min. Asterisks indicate samples for which data were not obtained. The procedures for measuring choline acetyltransferase activities and for cholinesterase staining are described elsewhere (17, 18). Section 238 was incubated with 1.8 mM acetylthiocholine for 2 hr at room temperature. The x's on section 238 mark artifacts; there is also a fold near the top of the section. The sections are from cat brain tissue block 022374L and, as indicated by their numbers, are located close to section 243 of Figures 2-5.

acids during certain special conditions, such as high noise exposure or drug treatment, may be examined.

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